THE ROLE OF OLFACTION IN HUMAN SOCIAL INTERACTIONS

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

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3rd January 2013

ABSTRACT

Olfactory signals are generally regarded as the oldest and most widespread form of communication across taxa. They can play an important role in the mediation of social interactions, reproductive status and warning of danger, whilst also signalling more fixed genetic factors such as the major histocompatability complex, which is linked to inbreeding avoidance. Little is known of the role odour might play in humans, although there is some indication that humans may be influenced by a family of androgen steroids called the 16-androstenes, produced in axillary odour. Here I aim to identify the role of androstene compounds in humans, as well as body odour as a whole, using two theoretical frameworks based on intersexual and intrasexual signalling.

Evidence is found to suggest androstadienone ("AND", a putative male pheromone) and male odour (a composite sample of axillary sweat) may be having a suppressive effect on males. Men exposed to AND feel less attractive and potentially behave less attractive too, as judged by third-party raters. In a physical performance test, male odour is found to have a similar suppressing effect, with exposure being linked to decreased men's performance in a cycling time trial and aspects of a 30-second cycling sprint test. In contrast, women exhibited stimulatory responses to the male odour, not AND.

An analytical assessment of male odour was carried out, in an effort to link androstene profiles to aspects of phenotypic quality and socially relevant traits. Cluster analysis revealed that men's odour profiles fell into two groups, which in turn could be explained by relationship status. In essence, single men have different compounds in their odour to men in a relationship. Furthermore, the odour of single men appears to be preferred by women. Exposure to solutions based on these two groups had sex specific effects on the receiver.

In conclusion, the results highlight the novel possibility of human male intra-sexual signalling, whilst providing the only empirical evidence of odour chemistry links with social function in humans; paving the way for further investigations in this field.

CONTENTS

ACKNOWLEDGEMENTS

I express great thanks to my primary supervisor Craig Roberts, for his guidance, support, and contagious enthusiasm for research that has never failed to inspire me in these four years. And also to my secondary supervisor, John Lycett, whose profound insight into human behaviour and evolutionary theory secured my interest from the start and who was always there to provide direction, encouragement, and invaluable advice when it was needed most.

For my time at Liverpool University: I owe great thanks to my rather brilliant colleagues and friends (Andy, Becky, Ewan, and Kath to name a few but not enough) from what used to be the department of Biological Sciences. It was, without a doubt, an excellent place to begin a PhD and I will forever look back on my time there with fondness. A special thanks to Tom Hayes for his help and advice with the preparation of the androstene solutions.

For my time at Stirling University: I thank the Psychology department and the School of Sport in particular for welcoming me into their research circles. To individuals at Scottish Institute for Sport, who kindly gave me space in their exceptional laboratory. I extend a special acknowledgement to Professor Kevin Tipton, who saw potential in my idea and gave me the opportunity to investigate it. Thanks to Mike, for teaching me everything there is to know about bikes and the Computrainer set-up (which, it turns out, is quite a lot), Tommy for all the help piloting, Chris for the support and sanity during data collection, and Frank Kelly for constructing the 'Smell Master-500' (odour headset) in Section Three, I expect a phone-call from Dragon's Den any day now.

I have had the privilege to work with various researchers who I owe great thanks to for the opportunity and the inspiration they provided: Dr Jan Christensen and Professor Patrizia d'Ettorre on the odour chemistry project, Dr Jan Havlicek and Dr Tamsin Saxton during the review on 16-androstenes, and Professor Kevin Tipton on the sport physiology work.

A PhD thesis relies on data, or participants, in my case. Therefore I feel I owe particular thanks to the hundreds of people who gave up some of their time to take part in any of my studies. A special

mention for the eight cyclists from Chapter Three, who showed an admirable Scottish determination to come to the lab each week circa 4:30am, to ride their bikes. And to the male odour donors, who could have quite easily reported me when I approached them asking for their armpit odours. Because of these friendly and enthusiastic volunteers, data collection with humans has, and always will be, my favourite thing to do.

I am very lucky to have had the support of my parents and three sisters throughout this process, so I thank them for putting up with me. I also thank my wonderful friends for the same reason, yet there are too many to mention, thank you.

And perhaps most importantly. I thank Tom, whose unwavering support and friendship quite literally kept me going.

Chapter One: An introduction to human olfactory signalling.

Chemical signals are generally regarded as the oldest and most widespread form of communication across taxa (Wilson, 1970). Communication via odour has benefits over other sensory modalities. For example, odour can go around barriers, be carried long distances in wind or water, and deliver a message in the absence of the signaller (Thornhill and Alcock, 1983). Furthermore, odour signals are not costly to produce and in some cases, may pose a smaller risk for the signaller (compared to elaborate visual and sound displays that may attract unwanted attention from predators). As a reflection of its importance in animal communication and behaviour, olfactory research has had a long history. A major breakthrough was the isolation of the first known pheromone, "Bombykol", which is produced by the female silk moth and detected by the male from up to 10km away (Butenandt et al., 1959). This was a groundbreaking study spanning two decades, where several hundred thousand female moths were reared to provide materials for the extensive bioassay performed to capture male behaviour responses.

The term "pheromone" was classically defined by Karlson and Luscher (1959) as a chemical(s) secreted by an animal that 'releases a specific reaction' in a member of the same species. Over the last 50 years this has been redefined into various subcategories, depending on the variety of behaviours and reactions they can elicit. For example, sex pheromones can be categorised as "primer" pheromones, which cause endocrinological changes (Wilson and Bossert, 1963) or "releaser" pheromones that cause immediate effects on the behaviour of the receiver. This categorisation is sometimes difficult, however, particularly when pheromones can fulfil both roles (e.g. the queen madibular pheromone in the honeybee (Aphis mellifera) (Winston and

Slessor, 1998)) or when considering complex behavioural interactions in mammals (Wyatt, 2003). In this thesis, the terms chemical signal and pheromone will be used interchangeably and, when the strength of the signal and its effects on the receiver is uncertain, "chemical cue" will be used.

This thesis examines the role of chemical signals in human social interactions, drawing on findings from animal research and incorporating an evolutionary approach to the design and framework of the project. The question of whether pheromones exist in humans remains a controversial one for a number of reasons. The human sense of smell, for example, is considered comparatively poor in relation to other animals (Schaal and Porter, 1991). In addition, humans spend an immense amount of time and money trying to mask and prevent natural body odours. Furthermore, almost sixty years of human pheromone research has yet to provide any definite answers to the questionable role of pheromones influencing human social interaction. This chapter aims to review research that has revealed the intriguing odour interactions that occur in humans. An introduction to the field of general odour research is then followed by an account of more specific compounds that some authors believe act as pheromones in humans.

1.1 Human body odour – a biological signal?

Humans are a primarily visual species and, consequently, their olfactory abilities have tended to be overlooked. Indeed, in The Descent of Man (1871), Darwin observed that "the sense of smell [in humans] is of extremely slight service, if any, even to the dark coloured races of men, in whom it is much more highly developed...". Here he is referring to observations made in native South American communities, where individuals were found to recognise people in the dark using odour alone (e.g. Houzeau, 1872; Humboldt and Sabine, 1849), which are, in fact, consistent with recent research suggesting that "identity signatures" in odour are more prominent in populations that do not bathe everyday (Marlowe, 2004). This somewhat insular statement is perhaps a testament to the times in which it was written, yet the general message of reduced olfactory importance in humans is prominent and worthy of note.

More than a century has passed since these early observations, bringing with it substantial advances in the science of human olfaction. More is now known about the physiology of our sensory systems and quantitative assessments of natural body odour have been made. With a greater number of odour-producing apocrine glands than our closely-related primate species and with the extensive development of axillary hair, Stoddart (1990) referred to humans as "the scented ape". We are now beginning to understand the importance of individual odour differences and the part they play in human social interactions.

1.1.1 The human olfactory system

Humans reportedly have about 10 million receptor cells in the olfactory epithelium; a small amount compared to the 230 million in dogs (Schaal and Porter, 1991). Humans also have a relatively low number of olfactory receptor genes (about 500-750 (Mombaerts, 1999)), most of which are not even functional and are present as pseudogenes (Glusman et al., 2001; Menashe et al., 2003; Zhang and Firestein, 2002).

In many mammals, there exists an area of specialised sensory receptors known as the vomeronasal organ (VNO). These cells are connected directly to the accessory olfactory bulb, allowing immediate detection and processing of pheromones. For more than ten years, the question of whether humans possess an analogous structure has been the subject of much debate. VNO 'pits' have been identified in the nasal cavity of adults, although their appearance is variable between individuals (but see Meredith, 2001 for a review; Moran et al., 1991; Stensaas et al., 1991). Moreover, no connection has been found between these cells and the olfactory bulb (Moran et al., 1991; Stensaas et al., 1991), suggesting that, even if this structure is present, it may be the vestigial remains of a once functional system.

While the VNO is essential in some animals for the perception and processing of pheromones, it is not a universal adaptation since many other animals use only their main olfactory system. For example in the domestic pig (Sus scrofa domesticus), blocking the female's VNO

does not affect her behavioural response to the male pheromone androstenone (Dorries et al., 1997). Therefore, simply because humans do not have a functioning VNO does not discount the possibility that chemosignalling occurs. That humans do, either consciously or sub-consciously, respond to odour cues has been revealed through technological advances in brain imaging techniques. Specifically Jacob et al. (2001b) have demonstrated brain activation in response to subliminal concentrations of body odour components. They reported brain activations in the cortices associated with processing not just olfactory stimuli, but emotional and attentional information as well (e.g. prefrontal cortex, hypothalamus, amygdale and visual cortex).

1.1.2 Odour as a cue for complementary genes

Human mate choice preferences are found to be partly correlated with the highly polymorphic genes of the major histocompatability complex (MHC). Protein products of the MHC play an essential role in immunological recognition, and so it is predicted that mating preferences should be for individuals with a dissimilar MHC. This is an adaptive strategy for two potential reasons: first, it could arise through a heterozygote advantage where the immunological resistance to pathogens and parasites of a female's offspring is enhanced (Apanius et al., 1997). Second, mating in this way may also reduce general inbreeding and hence increase average genomic heterozygosity, rather than heterozygosity specifically at the MHC (Potts et al., 1994; Potts and Wakeland, 1993).

Following studies in mice that demonstrate MHC-associated mate choice is mediated by odour cues (Yamazaki et al., 1976; Yamazaki et al., 1979), humans too have been demonstrated to show odour preferences for dissimilar MHC types (Wedekind and Furi, 1997; Wedekind et al., 1995b). In the North American Hutterites (a reproductively isolated community), couples are more MHC dissimilar than expected (Ober et al., 1997). However, other studies analysing the genotypes of established couples are inconsistent with their findings. For example, studies of

pairings in Japan (Ihara et al., 2000) and in South Amerindian tribes (Hedrick and Black, 1997) show no evidence of negative assortative mating for MHC. Equivocal findings may be due to differences in heterogeneity within the sample groups chosen. However there is also the possibility that the preference to mate disassortatively might be modulated by the benefits of choosing mates that score highly on other desirable traits such as dominance status (and see Roberts, 2009 for a review; Roberts and Gosling, 2003). Furthermore, evidence suggests that optimal mate choice preferences might be for intermediate MHC dissimilarity (Jacob et al., 2002b; Milinski, 2006; Reusch et al., 2001) rather than extremely dissimilar MHC. The task of identifying the genetic underpinning of mate choice preferences remains a complex, difficult, and, as yet, incomplete task.

Nonetheless, findings of human odour preferences that correlate with MHC dissimilarity have continued to emerge. Milinski and Wedekind (2001) found that preferences for component perfume ingredients correlated with a person's MHC, suggesting that perfumes may serve to enhance or complement individual variation in body odour, rather than mask it. This finding supports the biological function of perfume use, namely to amplify body odours, rather than the social perspective that perfumes function to hide or disguise socially undesirable odours. Furthermore, studies of the effect of hormonal contraceptives have reported reversed MHC preferences (Wedekind et al., 1995a) or significant preference shifts (Roberts et al., 2008) in favour of the odours of men with similar MHC types. Clearly, body odour seems to contain biologically relevant information that may contribute to mate choice outcomes, furthermore, detection might be easier in populations that do not habitually wash every day (Marlowe, 2004).

1.1.3 Menstrual synchrony

There is some evidence to suggest that women living in close proximity over several months will encounter menstrual synchrony (McClintock, 1971), which is suggested to be mediated by odour (McClintock, 1971; Stern and McClintock, 1998). McClintock's (1971) landmark study was based

on the menstrual cycle lengths of cohabiting women in a university dormitory, over a period of 8 months. However a later investigation found that menstrual synchrony did not occur only between close acquaintances, but more widely within the entire dormitory population, suggesting that other environmental factors could be in play (Little et al., 1989). Another study using the same methods and experimental design used in McClintock (1971) failed to find any evidence of menstrual synchrony, despite controlling for subjects with irregular cycles (Wilson et al., 1991). Yet many positive results have been reported and are consistent with McClintock's finding (Graham and McGrew, 1980; Quadagno et al., 1981; Weller and Weller, 1992, 1997, 1998; Weller et al., 1999a; 1999b). The variation in results could be attributed to the range of study populations chosen, as this varies from the positive results of cohabiting homosexual female couples (Weller and Weller, 1992) to basketball player teammates (Weller and Weller, 1995), where no synchrony was found. Furthermore, the majority of these studies have been criticized for their methods (Arden and Dye, 1998; Schank, 2001; Strassmann, 1999), pointing out, for example, the lack of control groups. Shank (2001) specifically highlighted how menstrual synchrony scores can vary depending on the calculation used, particularly when differing cycle lengths occur. There is also great concern for the credibility of results where the data are based on the memory of the participants rather than objective hormonal assessments.

Following exposure to axillary extracts taken from women at different stages of their cycles, Stern and McClintock (1998) were able to speed up or slow down the menstrual cycles of the women. Exposure to axillary extracts from women in their ovulatory phase significantly accelerated recipient's cycles, while extracts from pre-ovulatory women were found to slow them down. Male odour has also been demonstrated to have an effect on women's menstrual cycles, with experimental exposure to their odour causing cycles that were unusually long or short to become more regular (Cutler et al., 1986). Studies have also found that women who see men socially for more than three nights a week have significantly shorter menstrual cycles than women who do not (Cutler et al., 1980; McClintock, 1971; Veith et al., 1983). These latter results of cycle length manipulation are of particular interest, since they suggest that human odour is potentially

capable of altering neuroendocrine function which is a potentially useful applied outcome in terms of fertility treatments.

An evolutionary explanation for menstrual synchrony is not clear, as synchronised mating is a phenomenon more likely seen in seasonal breeding species. One advantage might be for females who might gain increased paternal investment: if a group of females are fertile at the same time, the operational sex ratio will be closer to 1:1, causing males to mate with fewer females at one time possibly leading to increased paternal investment (Wyatt, 2003). Another explanation could be that synchronization of births might present the advantage of communal rearing and/or a cohort of similar-aged individuals that, through play and other pro-social activity, positively influence behavioural development (Altmann, 1980; Kiltie, 1982; Lewis and Pusey, 1997). Alternatively, it might be a vestigial remain of a phenomenon that had a more adaptive function in primate ancestors (Roberts and Havlicek, 2012).

1.1.4 Odour and quality

Various studies have identified links between odour and variation in physical characteristics that reliably signal mate quality. Thornhill et al. (1998; 1999) reported that the odours of symmetrical males were rated more attractive by females in the fertile phase of their cycle, an effect they termed "the scent of symmetry" and is thought to be an honest indicator of genetic quality. In faces, it has been found that female preference for visual masculinity varies across the cycle (Penton-Voak et al., 1999) and with partnership status (Little et al., 2002). With this in mind, Havlicek et al. (2005) investigated whether the odour of dominant men is perceived by women in the same way. They found this was the case, since females rated the odour of dominant men as more attractive, particularly by those in the fertile phase of the menstrual cycle and in a long-term relationship. The putative links between human odour and underlying genetic quality are discussed in more detail in Section Three.

1.1.5 Odour cues of emotion and current state

Body odour may also vary within individuals, as it can cue more fluid factors such as emotional state, reproductive state in females, diet and, in some cases, health. Stress responses in all major taxa are associated with the release of chemical signals directed at surrounding conspecifics or predators (Fanselow, 1985; Suh et al., 2004; Wyatt, 2003; Zalaquett and Thiessen, 1991). For example, the European minnow (Phoxinus phoxinus) can induce a "fright response" almost instantly in other fish with its alarm pheromone, which is exhibited when skin damage occurs (von Frisch 1938 cited in Pfeiffer, 1974). Similarly, human odour samples taken from individuals experiencing anxiety have been found to have effects on behaviour and brain activity on others. Exposure to these anxiety signals have caused reduced perceptual acuity to social safety cues (Pause et al., 2004) whilst increasing ability to recognise social cues of danger (Mujica-Parodi et al., 2009; Zhou and Chen, 2009). Some reports have suggested that humans are able to detect the emotional state of a person by sniffing their axillary secretions (Ackerl et al., 2002; Chen and Haviland-Jones, 2000). There is also some indication that odours taken from other contexts, such as competitiveness or aggression, might influence physiological arousal (Adolph et al., 2010); these reports are addressed in Section Two.

In primates, fertile periods are often advertised in females with behavioural or morphological displays such as sexual swellings which are found to be attractive to males (Hrdy and Whitten, 1987). No such obvious displays are observed in humans, leading to the assumption that ovulation in females is concealed. Yet recent studies have revealed that changes in hedonic ratings of female odour can occur according to menstrual cycle phase. Early work showed that men prefer the odour of their partners when they are in the late follicular phase, compared to the luteal phase (Poran, 1995), yet it is unclear whether the participants were allowed to use perfume in this study (Singh and Bronstad, 2001). Despite methodological criticisms (detailed in Havlicek et al. 2006), Singh and Bronstad (2001) confirmed this finding, demonstrating that the odour of women is more attractive in the late follicular phase. The results of Kuukasjärvi et al. (2004) were consistent with this claim, adding further that the effect is non-existent in women who are on

hormonal contraception. In a recent study using a more sensitive within-subjects design, Havlicek et al. (2006) confirmed that both pleasantness and attractiveness ratings of axillary odours were found to peak at ovulatory periods.

It is perhaps unsurprising that diet might have significant consequences on human body odour; there are many anecdotal tales of certain pungent foods such as garlic, spices and vegetables altering natural odours. This assumption has very little empirical evidence however, with just one study demonstrating that red meat consumption correlates negatively with pleasantness ratings of body odour (Havlicek and Lenochova, 2006).

Odour may also cue certain medical conditions, such as diseases and metabolic disorders, yet there are few studies which have addressed this issue directly. More comprehensively studied in mice, odours of healthy males are preferred by females over those of males infected with parasitic coccidian protozoans (Eimeria vermiformis) (Kavaliers and Colwell, 1995), although this may not extend to full avoidance in mice infected with respiratory diseases (Penn et al., 1998). Wyatt (2003) reviewed the characteristic odours associated with certain human diseases, the use of which in medicine he describes as being a "lost art". Yet recent work has shown an ability of canines to detect certain cancers by smelling human urine (Gordon et al., 2008; Willis et al., 2004)

1.2 The 16-androstenes – putative human pheromones.

With the evidence reviewed so far, it is clear that human body odour may play some role in mate choice and other social interactions. How extensive and influential this role is still requires further investigation. In particular, knowledge of the specific chemical compounds that elicit these behaviours and underpin individual differences has not been mentioned. Such compounds are thought to include androstenes, a group of steroids present in axillary secretions that have been widely studied.

Androstenol, androstenone and androstadienone make up some of the androgen-derived steroids that have been found in human axillary secretions. Little is certain of their effects, specificity, or abundance within human interactions despite much research on each compound individually. Originally found to occur as a sex pheromone in male boars, androstenone was reported to elicit the lordosis posture in sows (Ahmad and Gower, 1968; Brophy and Gower, 1972; Signoret and du Mesnil du Buisson, 1961b). It was surprising then, to discover this compound, and others like it, in humans. Since their identification in human axillary odour some 30 years ago, the role of the 16-androstenes in human interactions remains inconclusive and there is continued reluctance to define them as 'pheromones'. Possible causes for this uncertainty are addressed throughout this thesis, particularly in Section One. For now, it seems worthwhile to introduce these compounds by describing their unique properties, highlighting certain aspects in their expression, detection and influence that have captured and sustained the interest of researchers over the years.

1.2.1 Androstene expression

Research into the variation of androstene expression between individuals is sparse, although there is some evidence to suggest that salivary levels of androstenone (Bird and Gower, 1983) and axillary odour levels of androstenol (Rennie et al., 1991) are higher in adult men compared to women. In addition, urinary androstenol concentrations are markedly higher in post-pubertal, compared to pre-pubertal, individuals (Cleveland and Savard, 1964). The results are not directly comparable due to the difference in substrates that were analysed, yet there is strong indication of a sexually dimorphic pattern of expression that emerges at puberty which is characteristic of sexually selected traits (Andersson, 1986). This was perhaps the starting point in a field of research that aimed to identify the 16-androstenes as sexually selected signals. Further discussions are detailed in Section Three, which addresses the quantification of 16-androstenes in human odour.

1.2.2 Variation in detection thresholds

There is substantial variation in the ability to detect the odour of androstene compounds, an intriguing property in odour research that has attracted much interest. Lundström et al. (2003b) revealed that women have lower thresholds to androstadienone (AND) than men. In the same study, it was suggested that individual AND sensitivity may be separate from general olfactory acuity, demonstrating a bimodal distribution in sensitivity to AND that did not correlate with thresholds to other odours. In a later study, Jacob et al. (2006) reported a multi-modal threshold distribution for AND, suggesting that thresholds are likely to be dependent upon individual variation in exposure history. This implies plasticity to androstene thresholds that can be environmentally influenced, at least to some extent. Indeed, sensitization to androstene compounds has been found to be possible following repeated odour exposure (Mainland et al., 2002; Wang et al., 2004; Wysocki et al., 1989; Yee and Wysocki, 2001).

Further variation in detection thresholds within individuals has been reported. Lundström et al. (2006) showed that women's sensitivity to AND was higher in the fertile phase than in the luteal phase of their menstrual cycles. This finding was evidenced by the lack of comparable change in responses to the control odours. Detection thresholds have also been compared across age groups, where peri-pubertal decreases in thresholds for androstenone (Dorries et al., 1989), AND (Hummel et al., 2005) and other malodorous components of human sweat (Chopra et al., 2008) have been reported in males but not females.

1.2.3 Hedonic perceptions

Similar to detection threshold properties, hedonic perceptions of the androstene odours are also found to vary both between and within individuals. Verbal labels range from these compounds being reminiscent of "strong urine" (Ohloff et al., 1983), "musky" (Jacob et al., 2002a) and "unpleasant" (Lundström et al., 2003b) odours. However, opinions are subject to change,

particularly in females where most studies of hedonics are focussed on changes in odour perception across the menstrual cycle. Hummel et al. (1991) investigated hedonic estimates of several non-androstene odorants, as well as androstenone, in women across their cycles. It was only with androstenone that trend analysis revealed a significant relationship between hedonic estimates and phases of the menstrual cycle, with more pleasant perceptions coinciding with ovulation. Similarly, Grammer (1993) reported that while females rated the main component of androstenone as unattractive, their response changed to a neutral one around the time of ovulation.

1.3 Human odour – an inter-sexual or intra-sexual signal?

Signals may evolve from chemicals that are already in use after being exaggerated, repeated and stereotyped in the process of ritualization (Huxley, 1914; Lorenz, 1966; Zahavi, 1977). For example, in various aquatic species, hormones released into the water through surface skin injuries now serve as alarm pheromones to surrounding conspecifics (e.g. Brown et al., 2000; Lawrence and Smith, 1989). In other cases, the driving forces behind the evolution of a signal are not always obvious. In humans, for example, potential sexually selected traits such as male voice may have evolved via two possible selection mechanisms. Firstly, lower pitched male voices may have arisen through female choice, where female preferences favour males with deeper voices (Collins, 2000; Oguchi and Kikuchi, 1997). Secondly, it could have been driven by intrasexual competition between males, where low voice pitch is associated with interpersonal power and deference relations (Benjamin, 1981; 1992; Gregory et al., 1993; Gregory, 1994; Puts et al., 2006). Female preferences for deeper male voices may still be explained by the intrasexual competition selection theory. If men's voices evolved as dominance signals, it could be the case that female preferences evolved secondarily in order to choose male voice that signal higher quality (i.e. those that have a lower-pitch).

The same could be said about the role of human odour in social interaction. Much of the research reviewed so far suggests that human odour has many properties in common with

established sexually selected traits. To return to The Descent of Man, Darwin (1871) suggested that sexually selected signals will have a number of properties in common. He stated that they will be expressed more in one sex, only develop in adulthood and their expression would be limited to mating contexts. Body odour appears to meet these criteria. As outlined above, it becomes more pronounced around puberty with expression of candidate pheromones appearing at this time; men are also found to be more odorous than women and many of the reported effects of human odour can be related to mate choice contexts. Yet, like the uncertainty surrounding the mechanisms behind sexual dimorphism in voice, it is unclear whether the main selection pressure acting upon it was intrasexual competition or female choice (intersexual). This fundamental issue is addressed throughout this thesis and, indeed, forms the basis of the theoretical framework around which the results are interpreted. Understanding the selection pressure acting on human odour interactions is paramount in understanding the role of odour in human interactions.

1.4 Thesis outline and objectives

The principal aim of this thesis is to examine the role of odour in human social interactions. The following data chapters are categorised into three separate, yet linked, sections. The first considers intersexual odour signalling in humans, the second highlights the possibility of intrasexual odour signalling, and finally an analytical assessment of odour is undertaken in Section Three. Each section falls within the central theme of the thesis (i.e. odour effects on human behaviour) but does so in different contexts. Within each section I report the findings of experiments relevant to the focus of the two signalling theories or the analytical approach.

Section One addresses the idea of human odour as an intersexual signal and will explore this with reference to a specific compound, namely androstadienone which is a putative human pheromone. Evidence is reviewed that suggests a primary role of the androstenes as male intersexual signals, whilst highlighting well-documented and more general examples from the animal literature. Methodological inconsistencies of previous studies that might explain

contradictory findings are discussed and addressed in Chapter Two. In addition, Chapter Two broadly examines the psychological and behavioural effects of androstadienone in men and women. In one study, a range of measured traits and behaviours that have hitherto been examined singly and independently in mate choice experiments are assessed. Amongst these are interpersonal attractiveness judgements, mood, self-perceived attractiveness and behaviour. By including a variety of measures and using both males and females as participants, it is hoped that the results from Chapter Two might reconcile previous equivocal findings in androstene research. Furthermore, a peripheral investigation into masking odour use is included. This addresses a concern with using the analgesic (and potentially adversely-affective) solution of clove oil as a masking odour by comparing the results of using a rose oil alternative.

Section Two extends the main finding reported in Section One (i.e. that male odour components may have a suppressive effect on males) and does so by considering the idea of human odour as a male intrasexual signal. The experimental aims of Chapters Three and Four are based on a recent study by Adolph et al. (2010) that reported increased physiological arousal after exposure to male odour from players competing in a sporting event. The studies in this section investigate whether the effect of what Adolph et al (2010) termed "competitive chemosignals" might extend beyond physiological arousal to an actual change in performance outcome. With the methodologies of Adolph et al. (2010) in mind, and without specific knowledge of socially relevant compounds and their concentrations in human odour, the studies in this section adopt the use of whole body odour samples. In this way, all biologically relevant information in odour is included.

To this end, Chapter Three examines the effect of male odour on an endurance-based exercise task using trained cyclists. The effect on performance was tested by comparing time trial results across odour conditions as well as competitive contexts; i.e. solitary or race testing sessions. Metabolic changes were also assessed in order to highlight any physiological changes. Chapter Four used a shorter, more strength-based performance task which was a quick and reliable way to test odour effects on a larger sample. In this study, anaerobic muscle power and fatigue were measured in an established procedure known as the Wingate test (Bar-Or, 1987). Females were

also included in this study to assess whether male odour signals have a sex-specific effect on performance.

Having adopted the use of androstadienone in Chapter Two and variations of whole male body odour in Chapters Three and Four, it seems necessary to reflect upon the research attempting to quantify the 16-androstenes in odour. Therefore, Section Three examines the issue that much of the (little) research in this area includes small-scale experiments with various methodological concerns. In Chapter Five, an analytical assessment of a large sample of male odour was performed using gas chromatography mass spectrometry. The resulting concentrations of androstene-type compounds were correlated with indices of phenotypic quality and other socially relevant factors. Results indicated that male odour profiles fell into two categories and the chemical content of each category could be explained by relationship status. Odours of single men contained more androstenol and androstenone-type compounds and were found to be rated more pleasant than the odours of partnered men.

The aim of Chapter Six was to test whether exposure to androstene mixtures, similar in composition to those found in Chapter Five, would elicit different responses in receivers. Various psychological measures, including attractiveness ratings, were taken and compared across odour conditions. In a sense, we have travelled full-circle, returning to an examination of androstene effects on attractiveness judgements and self perception, as seen in Chapter Two.

Finally, Chapter Seven summarizes these findings, and discusses their wider implications in human odour research. Evidence of intersexual signalling and intrasexual signalling in humans is reviewed, and the possibility of both occurring simultaneously is discussed.

SECTION ONE: THE 16-ANDROSTENES AND INTERSEXUAL ODOUR SIGNALLING IN HUMANS

The first section of this thesis presents evidence for intersexual odour signalling in humans, with particular focus on the 16-androstene steroids. It has been widely reported across taxa that male odour cues influence female mate choice decisions. Below, I provide a brief account of intersexual odour signalling in animals and how some of these examples may be applicable to humans. The evidence is categorised in terms of direct and indirect benefits to the receiver.

i. Pheromones as indicators of direct benefits

Some sexually selected male traits signal to the female that she will receive a direct benefit –for example through increased survival and/or fertilisation success- as a result of mating (Kirkpatrick and Ryan, 1991; Price et al., 1993) or through paternal investment. This kind of odour signal is common in insects where pheromones themselves will act as the resource benefit. For example, many insects pass on plant-derived anti-predator compounds during mating to protect the female and her eggs. The male odour signal in the North American tiger moth (Utetheisa ornatrix) is more or less attractive to the female depending on the amount of defensive plant alkaloids he is storing, which has a selective advantage for survival (Dussourd et al., 1991; Eisner and Meinwald, 1995). Pheromones may also indicate a male's ability to procure and maintain resources. For example, male American lobsters (Homarus americanus) fight for the largest nesting holes and those that are

able to hold their territory tend to have chemical signals most favoured by females (Atema, 1986, 1995).

In many animals, dominance might be conveyed or maintained through olfactory signalling (e.g. Johnston et al., 1997; Perret, 1992). Female mice prefer the urinary odour of dominant over subordinate males (Drickamer, 1989; Parmigiani, 1982, Hayashi, 1990). This is a widely studied phenomenon, and is one of the few cases where the chemicals responsible for the preference have been identified. At least four volatile chemicals are known to be attractive to females: a thiazole, a brevicomin and α and β farnesenes (Jemiolo et al 1985; 1991). More recently identified was the male-produced protein 'darcin' which elicits inherent sexual attraction in the female as well as stimulating a strong learned attraction to airbourne urinary odours of a specific male, and no others (Roberts et al., 2010).

If dominance carries with it the assumption of resource provision and social power, it might be argued that similar odour interactions occur in humans. Women are found to prefer the odour of more dominant men when they are most fertile (Havlicek et al., 2005). This could be partly due to the benefits that come with the investment and resources of a more dominant man.

ii. Pheromones as indicators of indirect benefits

Sexually selected signals may also correlate with aspects of 'good genes', giving indirect benefits to the female by improving viability of offspring. One example of such an indicator is the measure of fluctuating asymmetry (FA) which refers to small, random deviations from perfect symmetry on bilateral traits (e.g. wings, horns and markings). Deviations from symmetry are argued to result from an organism's inability to withstand environmental stress/perturbations and/or genetic problems during development (Møller and Swaddle, 1997). Females may, therefore, prefer highly symmetrical males as their symmetrical features indicate greater developmental stability; that is, they were able to maintain their health and survive despite the environmental and/or ontogenetic

stresses to which they were exposed (Møller and Thornhill, 1997). From this perspective, symmetry is held to be a good genes indicator that is difficult to fake. A number of studies have demonstrated a link between symmetry and achievement (e.g. racehorse success (Manning and Ockenden, 1994); perceptions of attractiveness (Penton-Voak et al., 2001); perceptions of health and personality (Fink et al., 2006)). Interestingly, Gangestad and Thornhill et al. (2005) reported that females, particularly those who were at the ovulatory phase of their menstrual cycles when tested, strongly preferred the scent of symmetrical males (relative to other less symmetrical males). The opposite effect, namely variation in males' preference for females, was absent, which is consistent with the claim by Thornhill and Gangestad (1999) that where olfaction might play a role in human sexual selection, effects are likely to be greater for females than for males.

Many studies have shown that female mice prefer the odour of males with a dissimilar major histocompatibility complex (MHC) which is a highly polymorphic region of genes that control immunological self/non-self recognition (Penn and Potts, 1998; Potts et al., 1991, 1994; Potts and Wakeland, 1993). This is believed to be an adaptive strategy for two potential reasons. First, benefits could arise through a heterozygote advantage where the immunological resistance of a female's offspring to pathogens and parasites is enhanced (Apanius et al., 1997). Second, MHC-dissimilar matings may also reduce general inbreeding and hence increase genomic heterozygosity of an individual's progeny (Potts et al., 1994; Potts and Wakeland, 1993).

Human female odour preferences also seem to point to disassortative mating for components of the MHC (for a review see Havlicek and Roberts, 2009). In women, the MHC influences both body odour and body odour preferences depending on stage of the menstrual cycle (Wedekind and Furi, 1997; Wedekind et al., 1995b). Furthermore, Milinski and Wedekind (2001) found that perfume preferences were correlated with a person's MHC, suggesting that perfumes may serve to enhance or complement individual variation in body odour, rather than mask it. Studies since then have supported this claim (Lenochova et al., 2012; see also Roberts and Havlicek, 2012 for a discussion of evolutionary pschology and perfume design).

iii. The 16-androstenes – intersexual signals?

How the 16-androstene steroids play a role in the evolutionary processes outlined above is unclear. A variety of effects have been reported that could be interpreted as intersexual signalling. For example, after exposure to androstenol, women reported more exchanges with males (Cowley and Brooksbank, 1991) and they also showed a preference to sit on seats in a waiting room that had been sprayed with androstenone, rather than on those that had not been sprayed (Kirk-Smith and Booth, 1980). More recently, androstadienone (AND) exposure has been reported to have numerous positive effects on mood and psychophysical arousal (e.g. Bensafi et al., 2004a; Bensafi et al., 2004b; Jacob et al., 2001a; Jacob and McClintock, 2000; Lundstrom and Olsson, 2005; Wyart et al., 2007).

Table I summarizes a large body of research that describes the specific psychological and behavioural effects of the 16-androstenes in humans, typically in females. A glance at the methodologies and compound choices and concentrations highlights the diversity of approaches used throughout studies in the last three decades. To take the example of compound choice, early studies commonly used androstenol and androstenone, potentially due to findings in pigs that show these compounds to act as a releaser pheromone in sexual activity (Signoret and du Mesnil du Buisson, 1961a). It was only later that researchers began to focus on AND, the compound more commonly seen in the literature today. The reason for this shift in interest is uncertain and, as discussed in Havlicek et al. (2010), seems to be based on fashionable trends rather than through the process of falsification. It could be said that this diversity in methodologies and the lack of logical progression between androstene studies gave rise to equivocal results. Hence there remains a great deal of reluctance to designate the name of 'pheromone' to the androstenes in humans.

The evidence outlined so far suggests that the androstenes are unique compounds that can have gender specific effects in certain circumstances. Yet there is a strong need now for studies that use ecologically valid and systematic approaches in order to tease apart their function. The

importance of ecological validity was highlighted by Saxton et al. (2008) who used face-to-face meetings in a speed dating event to gauge the effect of androstadienone on attractiveness ratings. Similarly, Lundström and Olsson (2005) reported AND-induced psychological arousal in women but only when the experimenter was male. In contrast, AND been found to have a positive effect (e.g. Jacob and McClintock, 2000) yet also no effect at all (Bensafi et al., 2003) on mood. Are these differences in results due to different contexts, compound concentrations and exposure methods? As Table I shows, Bensafi et al. used an AND concentration of 50mg and exposed the odours through jar smelling, whereas Jacob and McClintock used 250µM and applied a solution, with a clove oil mask, directly to the philtrum. Instead of searching for a new measure or trait that the androstenes may affect, more attention could be paid towards contradictory findings; comparing methodologies and compound use. It is this issue that Section One aims to address, by including a variety of measures in one investigation.

Chapter Two is an exploratory study, focussing on the psychological and behavioural effects of androstadienone, a relevant compound used in the current literature. The chapter brings together, in one study, a range of measured traits and behaviours that have hitherto been examined singly and independently in mate choice experiments (eg. attractiveness, mood, self perception and behaviour).

The study design also incorporates an investigation into masking odour use which has proven to be a controversial issue in androstene studies. The use of an additional odour, to mask the musky smell that some detect in androstenes, was considered a necessary precaution by Jacob and McClintock (2000). Although it could be argued that if sub-threshold concentrations are used, this should not be a cause for concern. Indeed, many of the earlier androstene studies did not use a masking odour (see Table I). Jacob and McClintock opted for the use of clove oil as a masking odour, yet this has since been queried on the grounds that it might create a complex odour mixture which might affect results; eugenol was offered as a similar, yet chemically simpler, alternative (Lundström et al., 2003a). Although alternatives have been suggested and used in recent studies (Lundström et al., 2003a; Olsson et al., 2006), a direct comparison of different masking odours and

their interaction with androstenes has not yet been addressed. In the following study, I compare clove with rose oil as a masking odour in a peripheral investigation alongside the main experiment.

By including a variety of measures, a comparison of masking odours and using both males and females as participants, it is hoped that Chapter Two findings might reconcile previous equivocal findings which were largely due to differences in methodologies.

Table I Summary of androstene studies which report potential intersexual signalling effects.

Study	Compound[a]	Conc./ Quantity	Target sex[b] (n)	Mask[c]	Exposure method	Measure	Main effect[b]
Positive results							
(Cowley *et al*., 1977)	AL	1mg/1 ml	F, M (183)	N	Surgical mask	Perception of others	F attributed more positive traits to others, M attributed more negative traits to others
(Cowley and Brooksbank, 1991)	AL	1mg	F(38), M(38)	N	Impregnated necklace	Opposite sex exchanges	F reported more exchanges with males
(Kirk-Smith and Booth, 1980)	AN	3.2µg, 16µg, 32µg	540 M and F	N	Sprayed chairs	Preference of seats	F preferred to sit on 3.2µg or 32µg sprayed seats.
(Saxton *et al*., 2008b)	AND	250µM	F (22, 19, 12)	Y	Upper lip	Attractiveness perception	F rated M as more attractive
(Hummer and McClintock, 2009)	AND	250µM	F(30), M (20)	Y	Upper lip	Perception of emotion	Raters more engaged to emotionally significant stimuli
(Jacob and McClintock, 2000) exp 1	AND	250µM	F(10), M(10)	N	Upper lip	Mood and alertness	Increased positive mood state in F
(Jacob and McClintock, 2000) exp 2	AND	250µM	F(31)	Y	Upper lip	Mood and alertness	Prevention of mood deterioration (modulatory effect).
(Lundström *et al*., 2003a)	AND	250µM	F(38, 40)	Y	Upper lip	Mood and concentration	Increased feelings of focus

(Bensafi *et al*., 2004a)	AND	50mg	F(36), M(36)	N	Jars	Mood, memory and autonomic function	Maintained positive mood and decreased memory of events in F
(Bensafi *et al*., 2004b)	AND	6250µM, 250µM	F(30), M(30)	N	Jars	Mood and autonomic function	6250µM solution increased positive mood and decreased negative mood in F only
(Lundstrom and Olsson, 2005)	AND	250µM	F(37)	Y	Upper lip	Mood and arousal	Increase in psychophysical arousal and mood when experimenter was M
(Villemure and Bushnell, 2007)	AND	250µM	F(48)	N	Jars	Pain thresholds and mood	Increased positive mood in F in the absence of pain
(Wyart *et al*., 2007)	AND	30mg	F(21)	N	Jars	Cortisol levels, mood and physiological arousal	Maintained positive mood and increased sexual arousal
(Jacob *et al*., 2002a)	AND, AL	250µM	F(18), M(19)	Y	Smelling swab/ upper lip	Mood	Reduced negative mood and increased positive mood
Negative results/ null findings							
(Black and Biron, 1982)	AL	1% in 95% ethanol	F(39), M(39)	N	Confederate's odour	Attractiveness perception	None
(Filsinger *et al*., 1984)	AN	1 mg	F(102), M(98)	N	Envelopes	Self and other perception	M rated M as more passive, F rated themselves as less sexy
(Filsinger *et al*., 1985)	AN, AL	1mg, 1mg	F(132), M(122)	N	Envelopes	Self and other perception	AL: M rated M more attractive. AN: F rated M and F less attractive
(Kirk-Smith *et al*., 1990)	AN	0.25mg	F(8), M(8)	N	Surgical mask	Perceptions of others	Rated others as 'less sexy'

(Cowley *et al*., 1980)	AL	1mg/1 ml	F(153)	N	Surgical mask	Mood during the menstrual cycle	Increased irritability with exposure during menses
(McCollough *et al*., 1981)	AL	2 second spray of Boar Mate™	F(161), M(59)	N	Surgical mask	Emotional resonsiveness	No effect
(Benton, 1982)	AL	150µg	F(18)	N	Upper lip	Mood during the menstrual cycle	F more submissive during middle of menstrual cycle
(Bensafi *et al*., 2003)	AND	50mg	F(12), M(12)	N	Jars	Mood	No effect
(Gustavson *et al*., 1987)	AL	2.5mg	480	N	Sprayed perspex	Choice of toilet cubicle	M avoided sprayed stalls, F showed no preference

[a] Compound abbreviations: AN refers to androstenone, AND to androstadienone, AL to androstenol
[b] Target sex refers to the individuals exposed to the compound (F = female, M = male)
[c] Masking odour: Yes/No (in all cases where yes, masking odour was eugenol/clove oil).

Chapter Two: The role of androstadienone in human mate choice – effects on attractiveness judgements and behaviour.

2.1 Introduction

Chapter Two describes a study designed to investigate the role of androstadienone (AND) in human mate choice. To do so, established theories of androstene effects on humans are tested, as well as some novel proposals. Here I investigate the effect of AND on a range of psychological aspects that can be explicitly and implicitly linked to mate choice decisions. Specifically, I examine the effect of AND on interpersonal attractiveness judgements, mood, self-perceived attractiveness and behaviour.

2.1.1 Interpersonal judgements

In some circumstances, androstenes have been found to affect interpersonal judgements. A summary of the various effects of the androstenes on psychological state can be found in Table I (Section 1, Introduction). Androstenol, for example, has been shown to increase positive opinions of others in women, but decrease them in men (Cowley *et al*., 1977). Androstenol exposure has been found to increase attractiveness ratings given to women by both men and women (Kirksmith *et al*., 1978). Both of these studies involved the rating of facial photographs in a laboratory setting, in contrast to a more ecologically valid study from Saxton *et al.* (2008a) where women were exposed AND or a control during several speed-dating events. In two out of the three dating events, women in the AND group rated the men as more attractive than the control groups.

While the above findings are consistent with what might be expected of a sexual function of androstenes in humans, other research seems to suggest otherwise. Black and Biron (1982) exposed participants to androstenol via the use of an opposite-sex confederate in the room, who was sprayed with the experimental odour. Subsequent ratings of the confederates revealed no difference between the androstenol group and the controls, yet Filsinger *et al.* (1985) later noted there was a statistical tendency for reduced attractiveness ratings of the androstenol-sprayed confederates. In similar tests with androstenone, Filsinger *et al*. (1984, 1985) reported that men rated other men as more passive with exposure to androstenone, while there was no change in women's ratings.

2.1.2 Mood changes

Early studies with androstenol indicate that exposure causes a rise in irritability for menstruating women (Cowley *et al*., 1980) and increased submissiveness mid-cycle (Benton, 1982). In contrast, androstenone exposure was found to increase alertness and excitability (Kirk-Smith *et al*., 1990).

For AND, the compound used in this study, results have been slightly different. Jacob and McClintock (2000) set the scene with a series of experiments assessing the effect of AND on mood, providing much of the methodological ideas used by future studies. In their experiment, Jacob and McClintock (2000) exposed men and women to AND, estratetraenol or a control whilst assessing their psychological state. Estratetraenol is a compound thought to be associated with females (Thysen *et al*., 1968) that has been described to have pheromone-like activities in primates (Laska *et al*., 2006). They found that AND and estratetraenol have sex-specific effects on aspects of positive mood. In both experimental conditions, women were reported to experience higher positive mood whereas men reported lower levels. The authors described this effect as modulatory, implying a more subtle impact of the androstenes as opposed to a release of stereotyped behaviours or emotions commonly seen in animal olfactory communication.

Consistent with this finding, Lundström *et al*. (2003a) state that women felt more focussed following application of AND, whereas Wyart *et al*. (2007) reported an increase in positive mood as well as sexual arousal. Furthermore, some reports suggest that female psychophysical and psychological responses to AND were only expressed when a male experimenter ran the study (Jacob *et al*., 2001a; Lundstrom and Olsson, 2005). Taken together, the studies on AND show early indications of a possible sex-pheromone function of AND in humans. However, given the contradictory results found using the very chemically similar compounds androstenol and androstenone, along with variation between studies in methodologies, it is clear more investigations are needed.

2.1.3 Self-perceived attractiveness

Subjective assessment of one's own attractiveness can in turn influence how one judges the attractiveness of others. For example, women who rate themselves as attractive tend to show a preference for faces of more masculine and symmetrical males (Little *et al*., 2001). Similar tendencies are also found in men, with the preference being for more feminine faces (Burriss *et al*., 2011). The effect of androstenes on self-perception and attractiveness judgments has been studied previously by Filsinger *et al*. (1984, 1985). In their first study, men and women were exposed to different odours while they completed a self-rating scale and rated a photograph of a target male. Participants were exposed to one of four odour conditions, including androstenone, the positive odour control of methyl anthranilate and the negative odour control of skatole, and a no odour control. Sex differences in the androstenone group were reported, with women rating themselves as less attractive and men rating the target male as more passive. Filsinger *et al*. (1985) followed the same procedure, with the addition of androstenol, a third musk-like odour and a female photograph to rate, alongside the original tasks from the previous study. Again, androstene compounds were reported to affect interpersonal judgements and self-ratings in a sex-dependent manner. For male participants, androstenol appeared to raise the attractiveness of the target male

and in the androstenol, androstenone and musk group, their self-ratings of sexiness were lower than those of the control group. For female participants, however, androstenol and androstenone lowered the attractiveness of the target male and had a varying effect on self-ratings depending on pill use and anosmia to androstenone.

2.1.4 Rationale

As the results of Filsinger *et al.* (1984) imply, androstenes in general do not seem to act in a predictable sex-pheromone manner. Why would putative male pheromones cause reduced intersexual attractiveness ratings in women? The authors attribute their somewhat contradictory results to a possible limitation in ecological validity, suggesting that the lab surroundings were not realistic enough to generate or trigger natural behaviour. Variation in results could also be caused by use of different compounds, odour presentation methods and concentrations used.

On the other hand, Jacob and McClintock's (2000) positive effect of AND on mood in women can be more easily applied to a sex-pheromone hypothesis, yet is not necessarily consistent with surrounding androstene literature. Other reports in the field indicate a rise in irritability and depression (Cowley *et al*., 1980) or even no effect on mood at all (Bensafi *et al*., 2003). Notwithstanding these doubts, a sex-specific pattern of androstene effects is evident in the majority of studies, providing scope for more investigation.

It seems at this stage, in order to gain a clearer understanding of androstene influences on human interactions, some of the previous results need to be revisited. Rather than searching for a new effect, this study attempts to draw together various conflicting findings in one investigation in order to minimise differences in methodologies and, possibly, thereby explain the equivocal findings reported above. This is not to say that the previous androstene studies have been inconsequential; on the contrary, they have provided a number of essential findings that form the basis of this study and indeed some components of this thesis, most notably that of the psychological effects of androstenes described in Filsinger *et al*. (1984; 1985) and Jacob and

McClintock (2000), which I aim to build upon in this study. Similarly, a measure of mood will give a more general idea of a person's psychological state. Each of these measures has been shown to be influenced by androstene exposure, and hence form some of the assessments made in this study.

This experiment also investigated the effect of AND on behaviour. Participants were videoed in the different experimental conditions and their recordings were later rated in the paradigm described below. They were asked to introduce themselves to a camera as if they were talking to a member of the opposite sex for the first time. Their video recordings were then shown alongside each other to a panel of third party raters in a forced-choice paradigm, where raters had to choose which version of the participant they thought was more attractive. A similar video assessment technique was used by Roberts *et al*. (2009) to quantify behavioural attractiveness. However the dual video presentation is, to my knowledge, a novel way of assessing behavioural changes in this field and was included to address the final question in this study: whether androstene-associated changes in mood and self-perception are also reflected in actual behaviour.

2.1.5 Use of masking odours in androstene research

When assessing psychological effects of androstenes, the use of masking odours has been argued to be a necessary measure (Jacob and McClintock, 2000). By masking the reported 'musky' odour of the androstenes, any psychological effects that are found can be ascribed to a possible pheromone-like influence, rather than a simple olfactory association (Jacob and McClintock, 2000). For this reason, Jacob and McClintock (2000) used clove oil as an odour mask in their study using AND and estratetraenol. They chose clove oil for a number of reasons. Firstly, is has been reported to have no effects on the surface potential of the vomeronasal organ (Montibloch and Grosser, 1991). Secondly, it is a botanical odour rather than a synthetic odour, although they did not state the significance behind this. Thirdly, it produces overall pleasant ratings and is generally not associated with perfumes or aftershave scents. Finally, it is used as a standard odour to

determine normal olfactory function (Dorries *et al*., 1989; Doty *et al*., 1995). To validate their decision, Jacob and McClintock (2000) tested a group of women in the mid-follicular phase of their menstrual cycle when olfactory acuity is in its peak (Doty, 1981). Participants were asked to provide descriptors for two solutions of clove oil with a propylene glycol carrier, one with 250μM of AND and one without. Descriptions were found to be almost identical, with a general consensus of a plant/food-like odour. When significant effects of AND were subsequently found in their mood and other psychological measures, reporting what they termed a 'modulatory effect', many succeeding studies followed the method of Jacob and McClintock (Jacob *et al*., 2002a; Jacob *et al*., 2001a; Jacob *et al*., 2001b; Jacob and McClintock, 2000; Lundström and Olsson, 2005; Saxton *et al*., 2008a; Saxton *et al*., 2008b). Lundström *et al.* (2003a) used eugenol as a masking odour, which is the main component of clove oil, to avoid the potential effect of using complex odour mixtures. Androstene studies using this mask have also reported significant psychological effects (Lundström *et al*., 2003a; Olsson *et al*., 2006), although it is unclear whether substitution of eugenol for clove oil would produce different results, as no direct comparison was made.

With some doubt emerging about the use of clove oil as an odour mask, and no research offering the comparison of using an alternative odour, a peripheral investigation into the effect of masking odours seemed a worthwhile extension to this study. The use of clove oil as an odour mask is queried on the grounds of firstly, there being a danger of creating a complex odour mixture (Lundström *et al*., 2003a). Secondly, it is a known analgesic and therefore may carry negative associations with participants, for example it may bring about the recall of memories of the dentist where clove oil is commonly used. To provide a comparison, half of the participants in this study were given AND in a clove mask, while the other half were given AND in a phenylethyl alcohol (PEA) masking odour. PEA was chosen as it is a near pure olfactory odorant with very little trigeminal component (Doty *et al*., 1978), and without any widespread negative associations. It is also commonly used in olfactory research when assessing olfactory sensitivity (Dorries *et al*., 1989; Doty *et al*., 1995). Due to its olfactory likeness to the fragrance of roses, those in the PEA

group were termed to be in the rose group. Any discrepancies in results for the two masking odours will have substantial effects on future methodologies.

2.2 Methods

2.2.1 Participants exposed to AND

Forty-eight participants aged between 18 and 30 (men: n=24, mean age± SD = 23.79±3.9; women: n=24, mean age± SD =22.08±3.37) were recruited from the Unilever consumer studies database. All participants were in good health and showed no evidence of major psychiatric or personality disorders, as demonstrated by a medical history questionnaire which was examined by a medical professional (Appendix I).

All participants were screened for normal olfactory sensitivity using the Burghart "12 Screening Test" Sniffin' Sticks (Hummel *et al*., 1997). This involved the identification of 12 every-day odours with the use of a multiple choice answer-sheet. Preliminary threshold data were obtained from the participants with the use of triangle tests during the pre-screen session. Solutions of AND, clove oil and rose were tested for detection rates. The triangle tests highlight those who have a low threshold to AND or exhibit a specific anosmia for the odours being used by asking the participant to identify the odd-one-out. No participants were excluded on the grounds of low thresholds or specific anosmia.

2.2.2 Ethical approval

Permission for the study was granted by the University of Liverpool Committee on Research Ethics as well as Unilever Research and Development Ethics Committee. All participants were given an information sheet before deciding to take part. They were told that the aim of the study was to investigate how odour affects human interactions and opinions, but were given no more

information regarding the different odour stimuli to be used. All participants signed an informed consent sheet before participation. Participants were reimbursed £25 for taking part in this experiment.

2.2.3 Odour stimuli

The experimental solution comprised of androste-4,16-dien-3-one (≥98% purity, Steraloids.com, CAS=794-58-9, MW=270.41) at a concentration of 250µM in a carrier solution of either 1% clove oil or 1% PEA in propylene glycol. Androstadienone was dissolved in the solution overnight on an automatic stirrer, transferred to a lidded container and kept in the refrigerator until 30 minutes before use, to allow it to return to room temperature. The control solution comprised of the 1% clove/PEA in proplylene glycol (≥99.5% purity, Sigma-Aldrich, CAS=57-55-6), with no AND.

2.2.4 Procedure

The experiment followed a within-subject, double-blind, randomised block design. Participants attended two experimental sessions, in each they were exposed either to an experimental solution (AND with masking odour) or a control (masking odour only). The order of solution presentation was counterbalanced across participants (half being exposed to the experimental solution on their first visit). The participants were also split in terms of masking odour used. Half were exposed to solutions with a clove oil mask, and half with a mask of rose.

Upon arrival, the odorous solution was applied to the participant's philtrum, using a cotton bud. On the basis of existing literature showing measurable effects of AND within 6-minutes post-exposure (Jacob and McClintock, 2000), I allowed for this time gap before the experimental tasks began. The concentration and presentation method of AND was used to allow comparison with existing literature (e.g. Jacob and McClintock, 2000; Jacob *et al.*, 2001; Lundström *et al.* 2003; Saxton *et al.* 2008a; 2008b).

2.2.4.1 Mood questionnaires

All participants completed a "Current Thoughts" questionnaire assessing present general mood and self esteem (Heatherton and Polivy, 1991) (Appendix II), followed by a series of questionnaires designed to measure other aspects of mood such as self-perceived dominance and attractiveness (Havlicek *et al*., 2005), physical attractiveness (Appendix III), dominance (Appendix IV), competence (Appendix V), extroversion (Appendix VI) scales (Goldberg, 1999) and Rosenberg's Self Esteem Scale (Rosenberg, 1965) (Appendix VII).

2.2.4.2 Attractiveness ratings

Participants then rated the attractiveness (on a scale of 1-7) of 12 males and 12 females, each in a picture and video format. Visual stimuli were presented in a randomized order on a java applet which recorded responses, as well as decision-making duration (the time it took for participants to make a rating).

2.2.4.3 Self-perceived attractiveness

In order to measure self-perceived attractiveness, Filsinger *et al*. (1984, 1985) used the Self-Rating Mood Scale from Bond and Lader (1974) with an added "sexy-not sexy" item. Other work on self ratings have used a simple Likert-type scale that participants rated themselves on (e.g. 1, low attractiveness; 3, average attractiveness; 5, high attractiveness) (Burriss *et al*., 2011; Little *et al*., 2001). To get a more accurate measure of self-ratings over time, Burriss *et al*. (2011) used the same scale twice and took an average from the two sessions. However, in light of recent evidence that has highlighted the importance of ecological validity in androstene experiments (Saxton *et al*., 2008a), I opted to use a more interactive method of measuring self perception. Here participants

viewed a set of 25 same-sex faces and a set of 25 opposite-sex faces. Whilst viewing the same-sex faces, they were asked "How attractive would you rate yourself compared to this person?" using a Likert-type scale with the anchors: "1= much less attractive, 4= about the same, 7=much more attractive". This was termed the same-sex comparison. For the opposite-sex comparison, they were asked "how likely is it that this person would find you attractive?" with the scale: "1= not at all likely, 4= neutral, 7= very likely". The mean scores were calculated from each of these tests to give two measures of self-rated attractiveness: a same-sex comparison and an opposite-sex comparison.

2.2.4.4 Photo and video recording

A neutral photograph and short video recording of each participant was then taken. During the video recording, they were asked to sit in a chair and introduce themselves to the camera as if they were meeting someone of the opposite sex for the first time. The chair was positioned 2m away from the camera, immediately in front of a plain black background in a windowless room with standardized overhead lighting. They were advised to talk for the amount of time that the experimenter was absent from the room, which they were told would be approximately one minute. The video clips were muted and edited to a duration of 15 s (starting from 5 s into the recording; videos of 15 s or less provide sufficient time to make accurate social and personality judgements (Ambady and Rosenthal, 1993; Babad, 2005; Dabbs *et al*., 2001) and encoded as 25 fps Windows Media Audio/Video files using MPEG-4 codec (Any Video Converter). Photographs were normalised on interpupillary distance and resampled to 400 × 480 pixels using Psychomorph (Tiddeman *et al*., 2001).

2.2.5 Third-party rating of photos and dynamic video stimuli

Sixty-eight participants aged between 19 and 24 (men: n=38, mean age± SE = 22.2±3.9; women: n=30, mean age± SE =23.5±3.6) were recruited from the University of Liverpool campus. Participants provided written consent before they were asked to view the photos and video clips from the experimental and control sessions. Due to the nature of the recruitment (i.e. time constraints of the volunteers), not all the participants could view the full set of stimuli. See Table 2.1 for a summary of rater numbers.

The stimuli were presented to the raters in a forced-choice test on a computer screen via a java applet. Each pair of faces or videos were of the same person recorded in the two experimental conditions: AND and control. Raters had to choose which photo or video they found more attractive by clicking on the scale below the preferred face.

Table 2.1. Summary of rater numbers. "Matched raters" refers to those who saw both the photo and the video of the exposed participant.

	Stimuli			
	Male		**Female**	
	Image	**Video**	**Image**	**Video**
All raters				
Female	24	26	29	24
Male	23	26	36	36
Matched raters only				
Female	22	22	24	24
Male	23	23	36	36

2.2.6 Analysis

All analyses were conducted with SPSS version 17.0. To test for differences between conditions, three multivariate repeated measures ANOVAs were used (Tabachnick and Fidell, 1996) with condition (AND, control) as a within-subjects factor and sex as the between-subjects measure; firstly with questionnaire scores as the within-subjects measures, then with attractiveness scores, and finally with self-perception scores. The self-perception data were categorized according to question type: same-sex comparison and opposite-sex comparison. These question categories were analysed together but treated as separate measures.

For the photo and video data, a one sample t-test was performed on the mean proportion of times a participant in their experimental condition was picked over their control condition. A test value of 0.5 was used. For completeness, only the data from raters who saw both the photo and video of the participant were used, although analysis with the entire data set showed no difference in results.

To analyse the effect of masking odour on human psychological aspects in general, an initial set of multivariate ANOVAs were performed on data from the control sessions, to avoid the potential interaction effect with AND. Here, masking odour and sex were used as fixed factors while questionnaire scores, interpersonal judgements and self perceived attractiveness were used in turn as the dependent variables. To search for an interaction effect with AND exposure, masking odour was added as a between subject factor in the above-mentioned repeated measures analysis.

2.3 Results

2.3.1 Comparison of masking odours – initial analysis

There were no discrepancies when comparing the use of clove and rose as masking odours (multivariate repeated measures ANOVA; mask/condition, $F(2, 42) = .171$, $P = .844$). Therefore the results from the two masking odour groups were analysed collectively.

2.3.2 Mood

Experimental condition had a significant effect on general mood and feelings of dominance, as measured by the current thoughts and psychological dominance questionnaires (F (1, 44) = 3.44, p = .041). This effect may be driven by non-significant, yet opposing, scores on the current thoughts scale, where a decrease occurred (F (1, 44) = 3.29, p = .077) and dominance scores where there was an increase (F (1, 44) = 3.22, P = .08) when exposed to AND, in both men and women. The remaining personality questionnaire scores were not significantly different between experimental conditions (F (6, 39) = 1.64, P > .1).

2.3.3 Interpersonal attractiveness judgements

For the attractiveness judgements of male and female videos, there was a near significant interaction effect between condition and sex (F (8, 33) = 2.21, P = .052). Univariate tests suggest that this result is driven by two significant comparisons: attractiveness scores of the female videos and the viewing duration of the male videos. For the female videos, participants were found less attractive by women whereas men gave a slightly higher score in the AND condition (F (1, 40) = 4.98, p = .031) (Fig. 2.1). For the male videos, women were found to take significantly longer, and men significantly less time, to make a rating (F (1, 40) = 6.51, p = .015), (Fig. 2.2). A paired samples t-test with female ratings showed that females also rated the males as less attractive under exposure to AND (t (23) = -2.16, p = .041, r = .411) (Fig. 2.3).

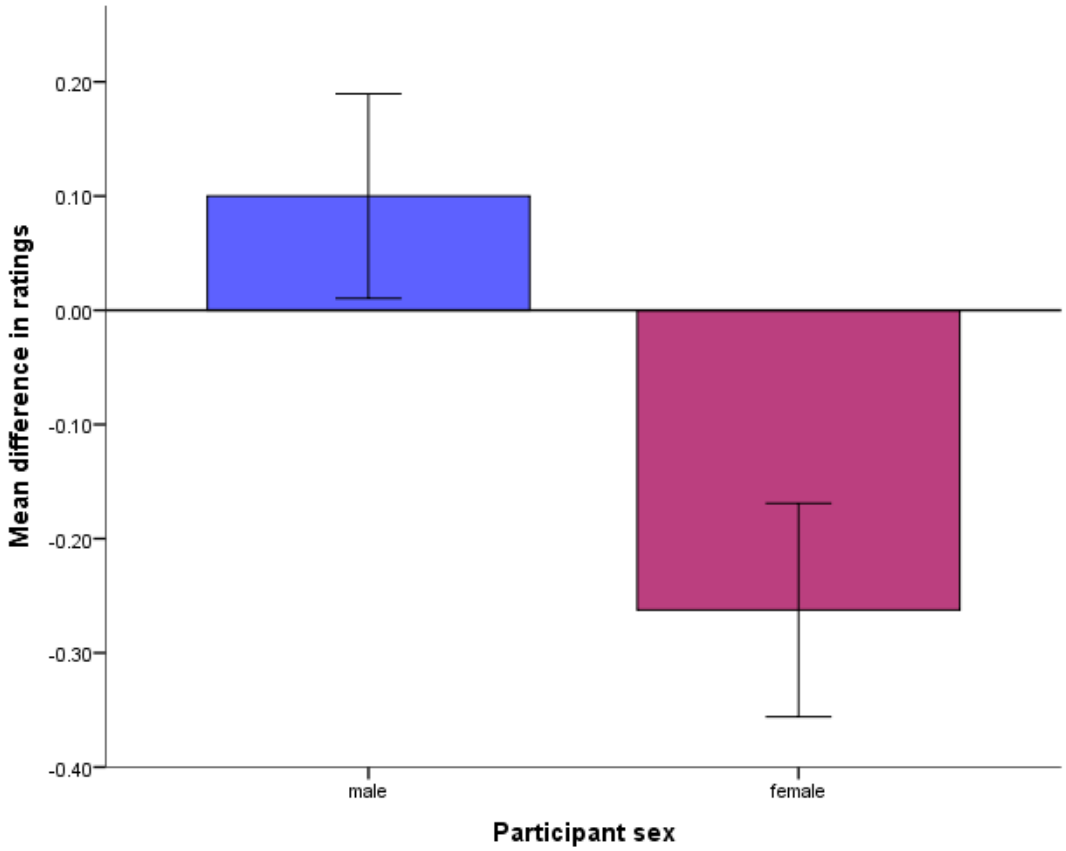

Figure 2.1 Mean difference (M±SE) in attractiveness scores, compared to the control, for female videos. There was a significant interaction effect between condition and sex, p = .031.

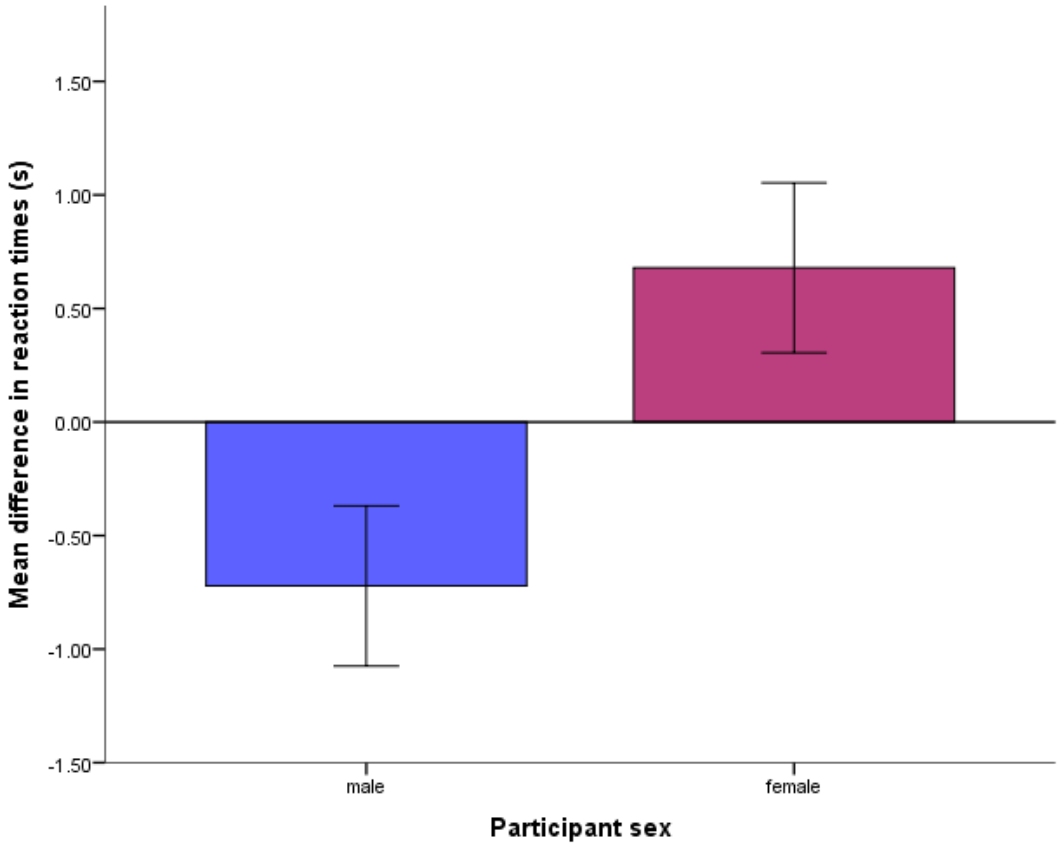

Figure 2.2 Mean difference (M±SE) in viewing duration times (in seconds), compared to the control, for male videos. There was a significant interaction between condition and sex, p =.015.

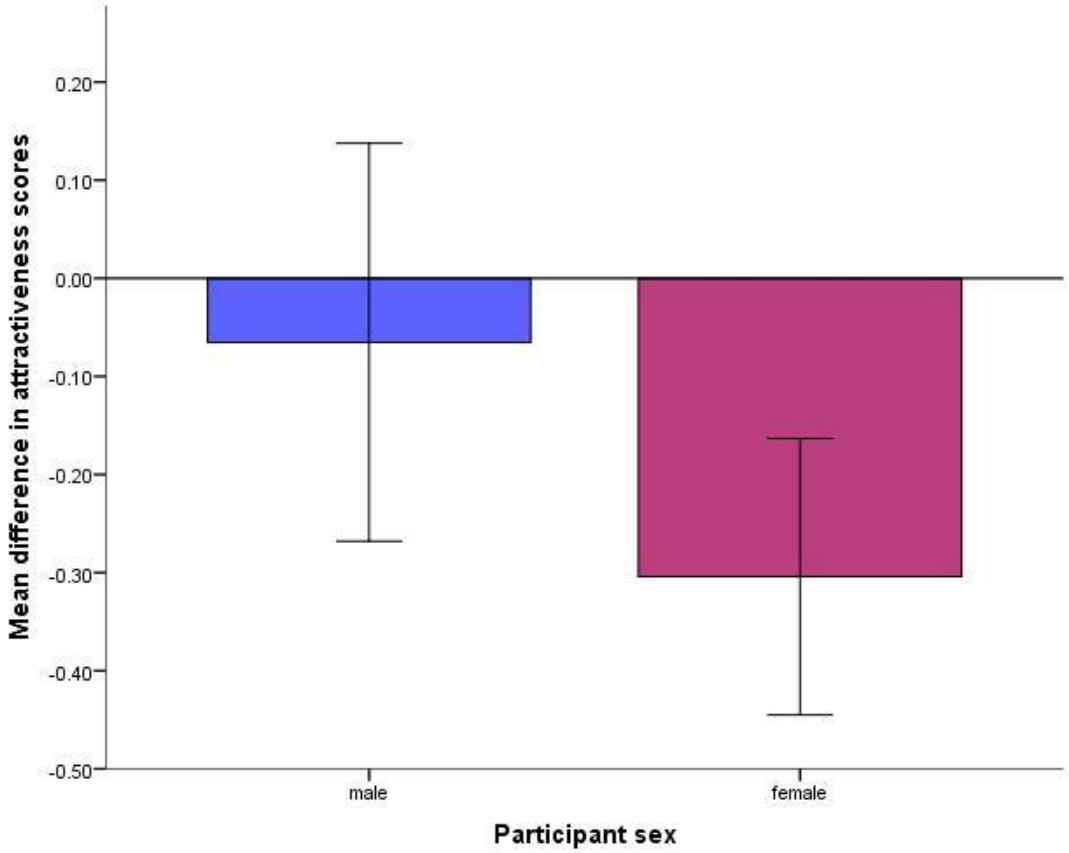

Figure 2.3 Mean difference (M±SE) in attractiveness scores, compared to the control, for male videos. There was a significant difference in females scores between conditions, as shown by a paired-samples t-test, p =.041.

2.3.4 Self-perceived attractiveness

When exposed to AND, participants rated themselves as less attractive than they did in the control condition (F (2, 36) = 3.961, P = .028). In the same-sex comparison (where participants were asked to give themselves an attractiveness rating in comparison to the image on screen) both men and women in the AND session rated themselves lower than they did in the control condition (F (1, 36) = 4.94, P = .032) (Fig. 2.4).

In the opposite-sex comparison (where they had to state how likely the person on screen would find them attractive), AND was only found to affect ratings of men, who again gave lower ratings than they did in the control (F (1, 36) = 4.52, P = .04) (Fig. 2.5).

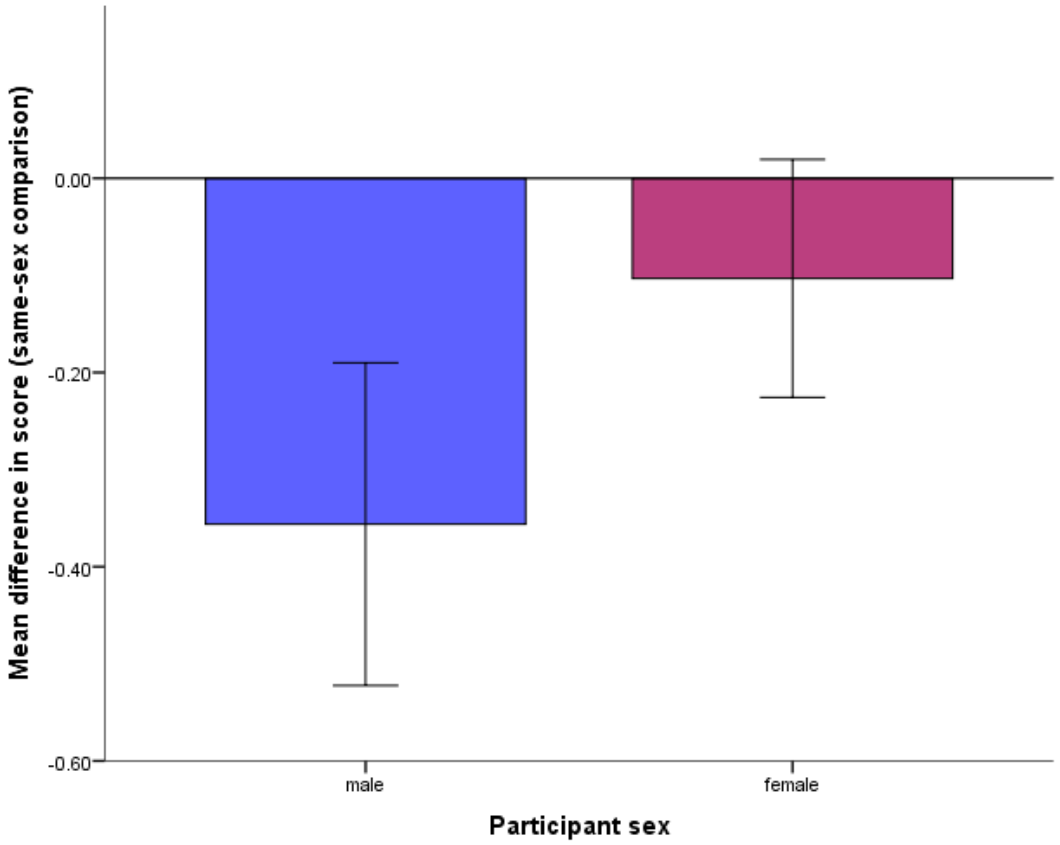

Figure 2.4 Mean difference (M±SE) in self rated attractiveness score, compared to the control, for the same sex comparison. Participants were asked: "How attractive would you rate yourself in comparison to this person?" There was a significant effect of condition, $p = .032$.

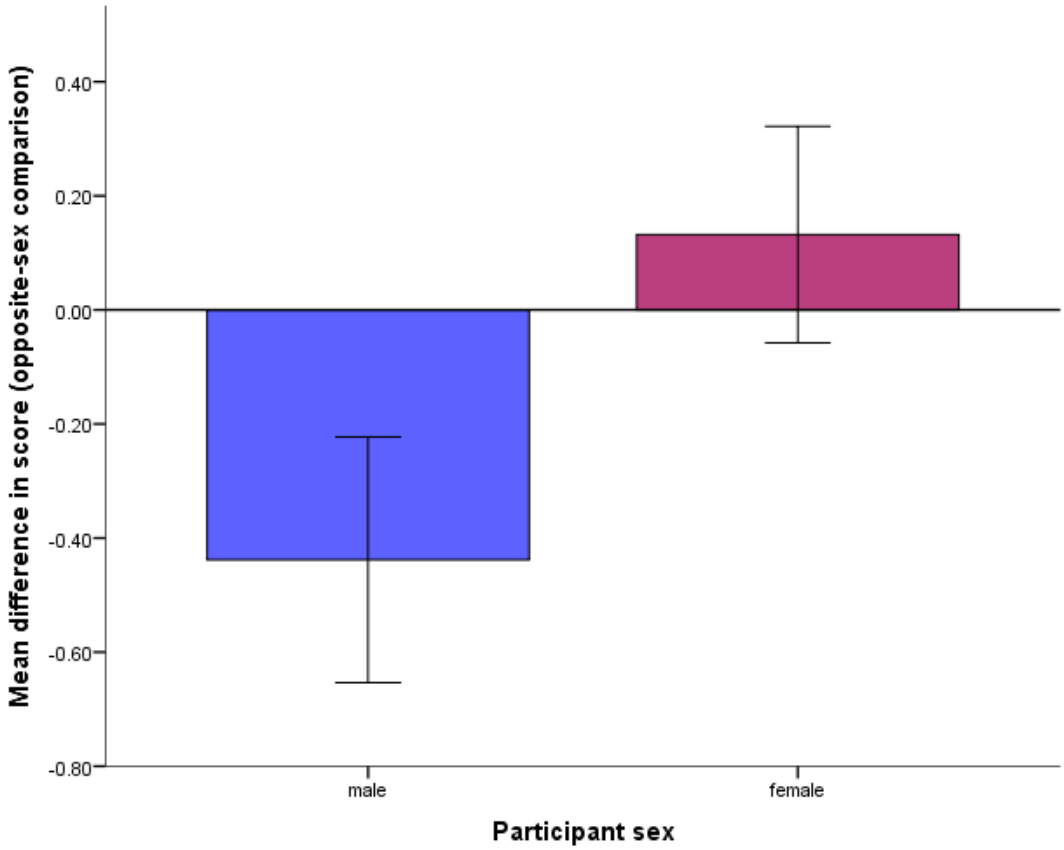

Figure 2.5 Mean difference in self rated scores (M±SE), compared to the control, for the opposite sex comparison. Participants were asked: "How likely is it that this person would find you attractive?". In men, there was a significant effect of condition, p = .04.

2.3.5 Dynamic stimuli ratings

An independent panel of raters viewed pairs of photos and videos of the participants, choosing which version of the person was more attractive. Analysis was performed on the matched raters' results (as seen in Table 2.1). On average, males were more likely to be preferred in their control condition video (M = .57, SE = .03) than in their AND condition video (M = .42, SE = .03), t (23) = -2.92, p = .016, r = .52) (Fig 2.6). There was no significant preference of condition for the photographs of participants (males: t (23) = -.50, p > .1, r = .1; females: t (23) = -.416, p > .1, r = .09) or of female video clips (t (23) = .55, p > .1, r = .11).

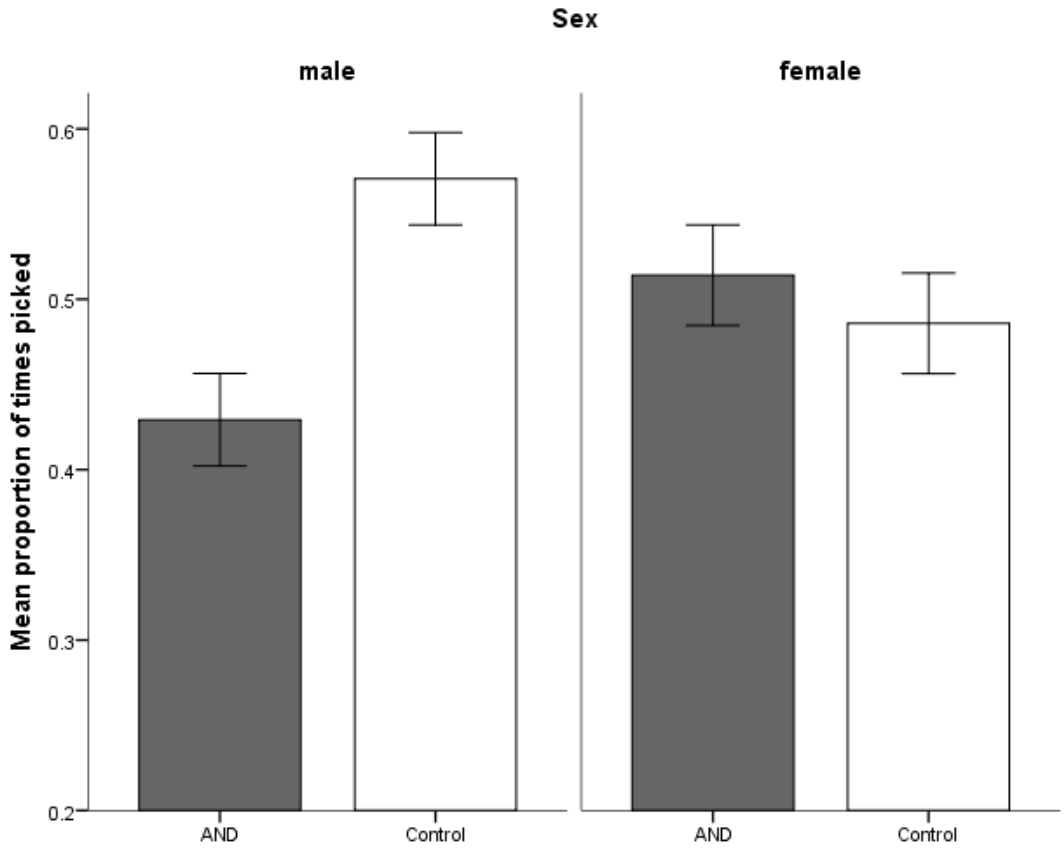

Figure 2.6. Mean proportion of times a video condition is picked (M±SE) (1.0 = 100% of the time). Males are more likely to be preferred in their control condition, as chosen by a set of third party raters. (t-test, p = .016).

2.3.6 The effect of two different masking odours

In the absence of AND exposure, masking odour did not effect mood and personality scales (F (6, 40) = 1.65, p = .16), interpersonal judgements (F(8, 37) = .76, p = .64), or self-perceived attractiveness (F (2, 41) = .86, p = .43). However, there were several significant interactions with AND exposure that are described below.

2.3.6.1 Mood and personality scales

As a between-subjects factor in the general analysis of the mood and personality questionnaires, masking odour was found to significantly interact with AND exposure (F (6, 37) = 2.38, p = .048). There was no interaction of condition and mask with participant sex (p = .106), yet univariate tests revealed significant results for the extraversion and competence scales. With exposure to AND, extraversion scores increased in the clove group and decreased, only in females, in the rose group (condition/mask/sex interaction: F (1, 42) = 4.53, p = .039) (Fig. 2.7). For the competence scale, scores were generally higher for males in the clove group and females in the rose group (condition/mask/sex interaction: F (1, 42) = 4.49, p = .04) (Fig. 2.8).

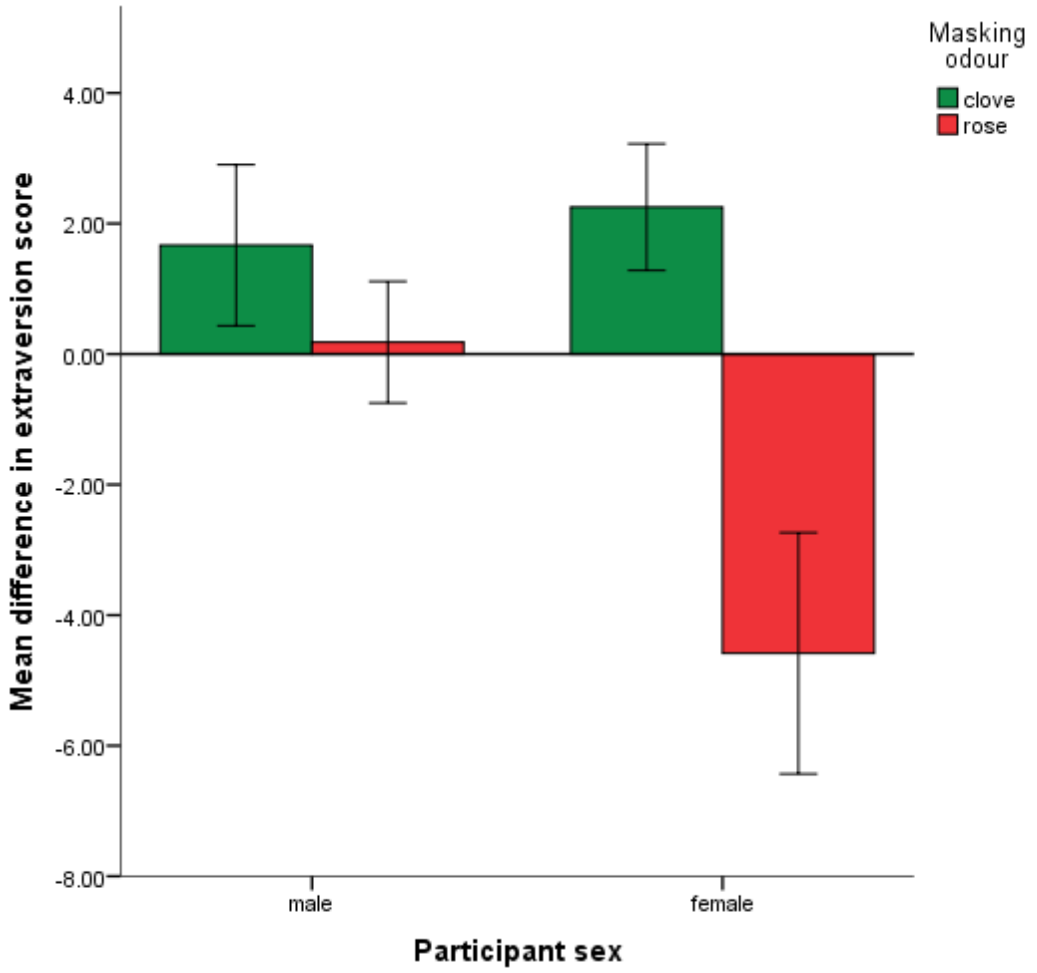

Figure 2.7. Mean difference in extraversion score (M±SE), compared to the control for both clove and rose masking odours. There was a significant interaction of condition, mask and participant sex (p = .039)

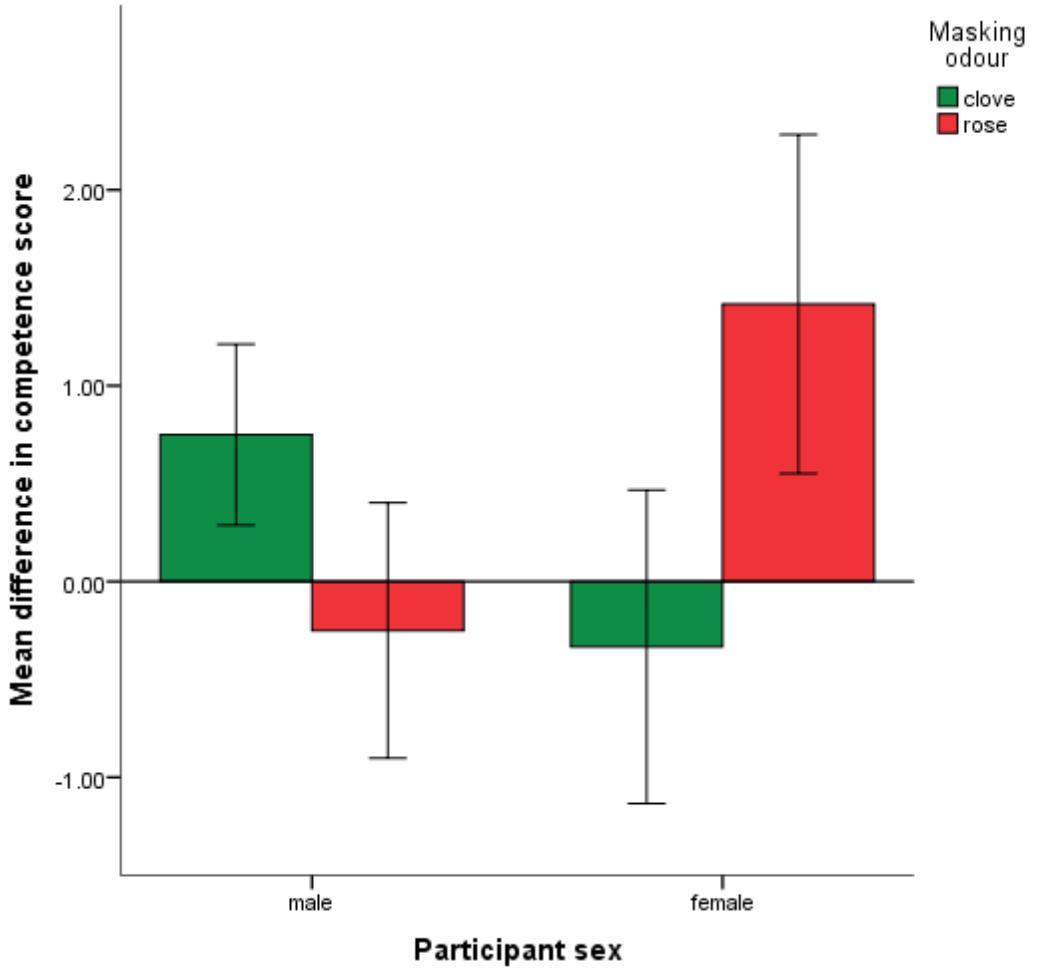

Figure 2.8. Mean difference in competence score (M±SE), compared to the control for both clove and rose masking odours. There was a significant interaction of condition, mask and participant sex (p = .04)

2.3.6.2 Interpersonal judgements of attractiveness

For the attractiveness judgements of pictures and videos, there was no interaction of masking odour with condition (F (4, 35) = 2.14, p = .565); nor was there an interaction of masking odour with condition and participant sex (F (4, 35) = 1.322, p = .281).

2.3.6.3 Self-perceived attractiveness

For self perceived attractiveness ratings, there was no significant effect of masking odour with condition (F (2, 34) = 2.14, P = .133), nor with condition and sex (F (2, 34) = 1.356, p = .271). Yet univariate tests reveal that there was a significant interaction of condition with mask for the same-sex comparison, where AND exposure was associated with lower scores in the clove group (F (1, 35) = 4.23, P = .047) (Fig. 2.9).

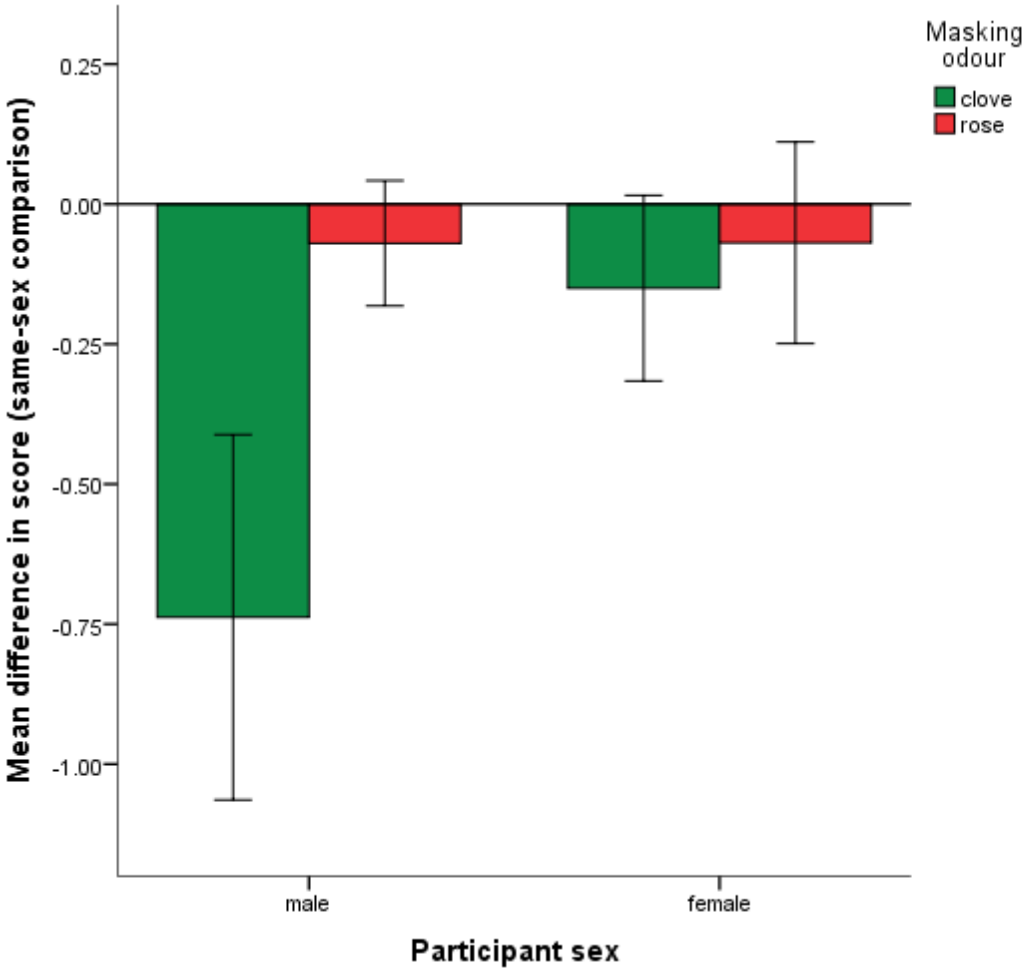

Figure 2.9. Mean difference in self perceived attractiveness score (M±SE), compared to the control, for both clove and rose masking odours. There was a significant interaction of condition and masking odour (p = .047)

2.4 Discussion

2.4.1 Mood and personality questionnaires

The current study suggests that AND causes mood to decrease and feelings of dominance to increase in both men and women. This is partly consistent with findings from Benton *et al.* (1982) and Cowley *et al*. (1980) where women exposed to androstenol showed increased feelings of submissiveness and irritability, respectively. Yet these studies explained their findings with respect to menstrual phase effects, a factor that was not included in the current study.

The present findings are not entirely consistent with other work on effects of AND on mood changes. Later studies have replicated Jacob and McClintock's (2000) finding, showing a reduction in negative mood in females (Jacob *et al*., 2002a), increased feelings of focus (Lundström *et al*., 2003a) and increased positive mood (Villemure and Bushnell, 2007). Why the present study and others before it (Cowley *et al.* 1980; Benton *et al.* 1982; Filsinger *et al.* 1984; 1985), found a negative effect of the androstenes on mood is unclear. One possible explanation could be to do with the varying contexts in the wide range of studies. AND effects in particular have been found to be susceptible to changes in social context. Jacob *et al*. (2001a) and Lundstrom *et al*. (2003a) found that AND had a positive effect on women's mood but a negative effect on males when the experimenter was male. Bensafi *et al*. (2004a) varied the emotional context of their experiment using films, reporting that AND had no effect on mood during a neutral film as opposed to a sad or arousing one. Similar reports of null findings in a neutral context have also been found by Bensafi *et al*. (2003) and Hummer and McClintock (2009). Given that the first task of the participants in the current study was to complete the mood and personality questionnaire, it could be suggested that at that point the context was predominantly neutral; and consequently, not socially relevant enough to elicit predicted effects.

Another point to consider is the questionnaire design that was used in this study. To measure general mood changes, a short scale instrument was chosen which is used often for assessments of current mental state (Heatherton and Polivy, 1991). This was given to the

participants alongside a series of personality questionnaires taken from The International Personality Item Pool (http://ipip.ori.org/). These were chosen to specifically assess socially relevant information such as dominance and self-rated attractiveness that can be linked to mate choice decisions. Due to the addition of these scales, the general mood questionnaire was perhaps not as thorough as that used by Jacob and McClintock (2000) who opted for a battery of tests originally designed to test the psychological effects of specific drugs during clinical trials and which have been recently adapted for use in psychological testing.

On balance, it is evident that androstene influences on mood are highly variable and depend on many other factors. However in this case, given that a slightly negative effect of AND on mood has been found despite the neutral surroundings that have confounded other experiments, this result might yet contribute to the field. An interesting finding in itself is evidence that AND affects male mood as well as females. This is in keeping with Jacob and McClintock (2000) who also found a decrease in men's mood; a result perhaps overshadowed by the more positive effect of AND in women that is more widely reported.

2.4.2 Interpersonal attractiveness judgements

There is some evidence in this study to suggest AND influences interpersonal judgements in humans. Exposure to AND was associated with decreased intrasexual ratings of attractiveness in women, but only in the video format. The implications of this are unclear, particularly as there was no significant difference in ratings for the self-perceived attractiveness test. That is to say, if women rate other women as less attractive, we may expect to see self-perceived attractiveness to improve. This result is discussed in more detail below, in section 2.4.3.

AND was also observed to be linked to a sex-specific change in the image viewing durations for the rating of male videos. Females exposed to AND took significantly longer to rate each male video than in their control condition; whereas the males were quicker. This suggests a more subtle effect of AND on mate choice decisions. The effect was not great enough to alter the

attractiveness judgements, but it seems to have made females consider the male for longer before a decision was made. This result could be construed as increased female choosiness, particularly as there was a statistical tendency for female ratings of the men to decrease. Taking into account the reduced time taken for males to rate female videos, this could also be described as a concentration effect, where AND might have a sex specific influence on feelings of focus. However if this is the case, the female result would therefore be inconsistent with Lundström *et al*. (2003a), who reported an *increase* in female focus with exposure to AND; not a decrease, which would be expected if women spent longer rating as a result of a negative effect on concentration.

These findings contrast with those from Saxton *et al*. (2008a), which imply that AND increases women's attractiveness judgements of males in a speed dating event. This could be due to a disparity in ecological validity between the studies, as subjects were not interacting with the males face-to-face as they did in Saxton *et al*. (2008a). Early studies with androstenol also found that exposure led to improved perceptions of others (Cowley *et al*., 1977) and increased attractiveness scores in both men and women (Kirksmith *et al*., 1978). As well as the compound differences between this study and the early work on androstenol, exposure methods have also evolved over time. Both Cowley *et al*. (1977) and Kirk-Smith *et al*. (1978) used surgical masks impregnated with odour; a method later criticized on the grounds that the participant is being exposed to much higher concentrations than those found in axillary odour and also through lack of social relevance to a mate choice study (Black and Biron, 1982).

An essential point to gather from these findings is the importance of the dynamic stimuli in this section of the experiment. With the few significant results emerging from the assessment of videos only, the necessity of more ecologically valid stimuli is heightened.

2.4.3 Self-perceived attractiveness

The results imply that AND has a negative effect on self-perceived attractiveness; a result consistent with Filsinger *et al*. (1984; 1985). In the same-sex comparison, where participants were

asked to rate their own attractiveness in comparison to members of the same sex, men rated themselves lower under exposure to AND. There was no significant difference in female scores, yet visual inspection of the data hints towards a trend for women to feel less attractive in comparison to others. In the opposite-sex comparison, men exhibited the same decline in score whilst female scores remained the same when exposed to AND.

Further investigation could look at whether these changes in self-perceived attractiveness are in turn influencing partner preference for symmetry and masculinity, as Little *et al*. (2001) and Burriss *et al*. (2011) show. It has been reported that men and women who rate themselves as highly attractive show a preference for symmetrical feminine or masculine faces, therefore if AND reduces the initial self-rating, it would be interesting to see whether this preference in others would reduce too.

2.4.4 Dynamic stimuli ratings

Bronson (1971) listed several conditions that should be met before the designation of pheromone could be applied. They included an experimental demonstration of the effects of the compound on behaviour, the isolation of such a compound or compound mixture, and lastly an experimental manipulation of behaviour with a synthesized mixture of the compounds.

To date, few studies have investigated the potential effect of the androstenes on true behaviour, whilst those that have report results that have proved to be largely inconsistent. For example in one study, females have been found to prefer to sit in seats sprayed with androstenone (Kirksmith *et al*., 1978), whereas in another they showed no preference (Gustavson *et al*., 1987). In this experiment we addressed this issue by making a video recording of the participants and asking a panel of third-party raters to assess them for attractiveness. By displaying the participants in both of the experimental conditions, the raters were able to make a direct comparison of behaviour, which is a novel approach to measuring behavioural changes in this field. Results showed that, on average, males were preferred in the control condition, while no difference

between conditions was observed for the female participants. Furthermore, there was no significant preference during the photo comparison task, implying that any preferences in the videos were due to a difference in the participant's behaviour between conditions rather than a difference in general appearance. This suggests that not only does AND have a negative effect on male self-perception (see above), but also on their behaviour as well, causing them to project themselves to others in a less attractive way.

2.4.5 Masking odour

It was hypothesised that the function of clove oil as an analgesic, particularly in dentistry, may hold negative connotations for participants and hence interfere with mood ratings. Indeed, masking odour was found to significantly interact with AND exposure during assessment of mood. However, a closer inspection of the data suggests this may be a coincidental result. The significance was largely driven by the extraversion and competence scales, where extraversion scores increased in the clove group and decreased in the rose group under AND exposure. For the competence scale, scores were generally higher in the clove group.

There was no significant interaction between masking odour and condition for the self-perceived attractiveness tests, nor for attractiveness ratings of the picture and videos. However, univariate analyses reveal that for the same-sex comparison in the AND condition, those in the clove group rated themselves lower than those in the rose group. Could this be due to the negative connotations of clove hypothesised to influence psychological state? This seems unlikely, given that extraversion and competence scores show a general trend to be higher in the clove group.

In summary, although masking odour seemed to interact with AND function to some extent, the interactions were sporadic and at times contradictory. Furthermore, many of the interactions of masking odour were with measures that did not prove significant in the main analysis of AND function, e.g. competence and extraversions scales, ratings of female pictures and

reaction times for female videos. Consistent with these findings, an independent analysis comparing clove and rose in the absence of AND, revealed no difference between the odours.

It may also be the case that masking odours interfere with androstene function physiologically rather than psychologically. It is hypothesised that trigeminal components of odours will affect experimental outcomes. Boyle *et al.* (2006) reported that AND perception is partially mediated by trigeminal activation. As most odours have a trigeminal component, with only a few being found without one (Doty *et al*., 1978), it is possible that the masking odour chosen may affect the function of AND by contributing to the trigeminal activation. With this in mind, future work planning to use masking odours may well incorporate trigeminal data if the opportunity and the resources arise.

A final question remains: whether the use of masking odours is necessary at all when using sub-threshold concentrations of AND. Further studies could incorporate the use of unmasked AND solutions to assess the need for masking odour and the potential interference it causes.

2.5 Final conclusions

The results from this study indicate that the effect of AND on humans is dependent on gender and context. Findings suggest that for men and women, AND might decrease mood and increase feelings of dominance, while, in men only, self-perceived attractiveness is found to drop with AND exposure. What is more, this reduction in self perceived attractiveness seems to be translated into a behavioural change in males. It seems that not only does AND cause men to feel less attractive, but it appears to make them behave less attractive in a perceivable way; consequently reducing their attractiveness to others.

The prevalence of an effect in males throughout this study is interesting to note. The outcomes have generally been negative, potentially indicative of a suppressive effect of AND in

males. This function of odour is reminiscent of that found in other species, where male dominance is transmitted as an odour signal, warding off competitors.

Taken together, these results offer three points of interest to the area of human chemosignal research. Firstly, they highlight an effect in males that many have not addressed previously. Secondly, they suggest that the modulatory effect of AND may in fact be translated into a behaviour outcome in males. And finally, they support the notion that AND-induced changes may be context-dependent; revealing that another context, such as male-male competition may be relevant. In the next chapter, I test this idea by examining the effect of male odour on the sporting performance of male cyclists.

The 16-androstenes and intersexual odour signalling in humans: general discussion

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This section of the thesis considered the evidence for intersexual odour signalling in humans, with a particular focus on the 16-androstenes. A large number of studies have examined the effects of androstenol, androstenone, and androstadienone on aspects of psychological function, with varying and often contradictory results (summarized in Table I, Section One). The equivocacy of research findings can be at least partly explained by a number of factors that varied across the different studies: the difference in compounds, concentrations, and exposure methods are some of the explanations discussed in Chapter Two. There is also a concern that few studies have controlled for menstrual cycle stage or pill use; both are factors which are found to alter female preferences for visual and olfactory mate choice cues (e.g. Penton-Voak *et al.*, 1999; Havlicek *et al*., 2005). For example, Havlicek *et al.* (2005) found that women only found the odour of more dominant males attractive during the fertile phase of their menstrual cycle, whereas women on the pill showed no preference. This highlights the need to include hormonal information (such as menstrual cycle phase and pill use) in studies where female mate choice preferences are recorded, and perhaps goes some way in explaining previous inconsistent findings.

The aim of the empirical study reported in this section was to test the effect of AND on a variety of psychological aspects that have hitherto been assessed independently, including mood, self-perceived attractiveness, and behaviour. The results provided little evidence of a sex-pheromone-like effect of AND in women. Exposure to AND was not found to affect female judgements of attractiveness, although there was a slight trend for female self-perceived

attractiveness to be lower compared to the control condition. It was found however that females exposed to AND took longer to make a rating when viewing male videos, a response that could be construed as choosiness. There was a tendency for mood to decrease, although this was not significant. Together, these findings are at odds with those that find a positive effect of AND on female mood (Bensafi *et al*., 2004a; 2004b; Jacob *et al*., 2002a; Jacob and McClintock, 2000; Lundstrom and Olsson, 2005; Villemure and Bushnell, 2007; Wyart *et al*., 2007) and women's attractiveness judgements of men (Saxton *et al*., 2008b).

Perhaps the most intriguing finding was that AND appeared to be having a suppressive effect on male participants. It seems that not only does AND cause men to feel less attractive, but it appears to make them behave in a less attractive way as rated by third-party observers which, presumably, will consequently reduce their perceived attractiveness to others. This is consistent with earlier research where androstenes were reported to have negative effects on male self perception (Filsinger *et al*., 1985), mood (Jacob and McClintock, 2000), and behaviour (Gustavson *et al*., 1987; Kirksmith *et al*., 1978). It is also one of the main functions of odour communication in animals, where chemical cues of dominance and status help mediate social interactions (for a review of animal pheromones, see Wyatt, 2003).

An investigation of masking odour was also outlined in this section. Following growing concerns around the use of clove oil as a masking odour (described in Chapter Two), I decided to include rose oil so a comparison of odours could be made. There was little difference in the results between the two odours.

On balance, the study in Section One suggests a suppressive effect of chemosignals in males that provide scope for further testing. Next, the direction of the thesis will change to accommodate this finding, by investigating the role of odour in intrasexual competition.

SECTION TWO: HUMAN MALE ODOUR AS AN INTRA-SEXUAL SIGNAL

In the previous section, I explored how putative pheromones influenced judgements related to mate choice. In this section, I present two investigations into potential effects of male odour on intrasexual competition. Following the findings reported in Section One, that male odour components have a suppressant effect on males, this section will change focus from mate choice interactions and consider physical performance in male-male competitive contexts, using the context of sporting competition.

Across taxa, male odour is known to convey information about, *inter alia*, individual dominance and social status to potential competitors. The odour signals in the male house mouse and their scent marking interactions are particularly well studied. In mice, resource holders and dominant males have been reported to scent mark more (Miller *et al*. 1987; Rozenfeld *et al.* 1987; Hurst 1990; Allen *et al*. 1999). Males will therefore avoid scent marked substrates, particularly when they are of low competitive ability (Gosling *et al*. 1996 a and b) or when the scent is from a dominant male (Jones and Nowell, 1989; Hurst, 1993). Here they might be avoiding a fight with a male who may be the territory owner (Gosling and McKay, 1990)(Hurst et al. 1994). Similar suppressant effects of male odour have been reported in the lesser mouse lemur (*Microcebus murinus*), where the urinary pheromone of dominant males will repress testosterone levels, and hence sexual activity, in subordinate males (Perret, 1992).

Possible odour-induced suppressing effects have also been identified in the previous chapter, suggesting that competitive odour signalling between males could be occurring in humans.

The data presented in Chapter Two indicated a decrease in male ratings of their self-perceived attractiveness relative to males presented in target stimuli, as well as a reduction in attractive behaviour following exposure to androstadienone.

Section Two will therefore employ a new context to test odour interactions in humans, following a recent study by Adolph *et al*. (2010). After discovering that humans produce and respond to chemosensory anxiety signals (Pause *et al*., 2004; Pause *et al*., 2003), Adolph and colleagues reported that competition too, can influence human odour (Adolph *et al*., 2010). They demonstrated that men and women displayed higher skin conductance responses when they were exposed to body odour collected during a competitive context (a badminton tournament match) compared to odour with the same intensity taken from a control condition that involved a solitary exercise control. The experiments that I report in this section examine if this psychophysical effect can also be expressed in terms of a performance output. Here I test the hypothesis that competitive chemosignals suppress physical performance in males.

Chapter Three attempts to answer this question in an endurance-based exercise task using trained cyclists. The effect of competitive odour signals on performance was tested by comparing time trial results across odour conditions as well as competitive contexts; i.e. solitary or race testing sessions. As well as recording time trial results, metabolic measures via gas calorimetry were also taken in order to highlight further physiological changes. The study in Chapter Four uses a shorter, more strength-based performance task which was a quick and reliable way to test odour effects on a larger sample. In this study, anaerobic muscle power and fatigue were measured in an established procedure known as the Wingate Test (Bar-Or, 1987). Females were also included in this study to assess whether male odour signals have a sex specific effect on performance output.

With the methodologies of Adolph *et al.* in mind, and without specific knowledge of socially relevant compounds and their concentrations in human odour, the studies in this section adopt the use of whole body odour samples. In this way, all biologically relevant information in odour is included.

Chapter Three: The effect of male body odour on physical performance: endurance in males.

3.1 Introduction

In this chapter I describe a study that examines the effect of contextual male odour on physical performance. Following evidence from the previous chapter that points towards an intrasexual competitive odour signalling system in men, it was decided to test this idea directly in a sporting environment where competitiveness can be easily established.

There is recent evidence to show that human emotional state can be perceived through body odour. Odour samples collected from individuals whilst watching emotion-inducing films have been shown to exhibit recognisable olfactory cues of fear and aggression (Ackerl *et al*., 2002) as well as mood states of happiness (Chen and Haviland-Jones, 2000). These findings are based on subjective ratings of worn cotton pads or T-shirts; further research into the effect on the perceiver suggests that fear cues enhance cognitive performance and facial fear recognition in the recipient (Chen *et al*., 2006; Zhou and Chen, 2009). Fear cues derived by more active means such as skydiving have been shown to cause amygdala activation as well as enhanced emotion perception in others (Mujica-Parodi *et al*., 2009). Similarly, chemosensory cues of anxiety have been found to affect facial emotion processing in females (Pause *et al*. 2004) and increase acoustic startle reflex (Pause *et al*., 2009). This is consistent with neuronal research which shows that olfactory anxiety cues activate the fusiform gyrus, an area of the brain involved in the processing of emotional stimuli.

In a later study, Adolph *et al.* (2010) demonstrated that competitiveness may also be revealed in human odour. This is already a well-documented trait in animals, where it is common for males to communicate their dominance to others in competitive contexts (Roberts *et al*., 2001). To collect samples from a competitive context, axillary pads were worn by males taking part in a badminton tournament. For a neutral context control sample, the same individuals were asked to complete a jogging workout with a level of effort similar to that of a badminton match. Samples were then pooled according to context, homogenised and divided into small portions to be presented to a set of raters via an olfactometer. As the odour was presented, skin conductance responses (SCR) were recorded. Results showed that the SCR was larger in the perceiver when they were exposed to the competitive odour compared to the control odour. Furthermore, SCR was found to be dependent on the trait anxiety scores of the raters, as measured by the Social interaction and Anxiety Schedule: more socially anxious individuals experienced greater increases in SCR when exposed to the competitive odour. Together these findings are consistent with current research into olfactory signals of anxiety and fear, and their ability to alter emotional perceptions and brain activation, particularly in highly socially anxious individuals. They also demonstrate that competitiveness, a socially relevant emotional state, can be revealed in odour in a way that causes detectable physiological effects in the perceiver.

The aim of this chapter is to build on Adolph *et al.*'s (2010) finding, investigating the effect of competitive chemosignals on behaviour as well as other physiological aspects such as a metabolic measure of energy expenditure. In keeping with the context of competition, this study investigates the effect of competitive chemosignals on sporting performance; a contextually relevant behaviour measure. Performance in this case is measured in male cyclists whilst they complete a 20km time trial. This experimental protocol was devised to assess the more long-term consequences of odour signalling such as endurance and metabolic changes during exercise.

The measurement of performance is one of the most important aims in sport science and physiology research, leading to much investigation into the correct protocol to test for this. For this study, a simple protocol was needed which was reliable, sensitive and possible to complete

without any intervening social interactions. Hence, a cyclist time trial was used as the experimental protocol. Cycling is a sport with simple techniques and is easily replicated in a laboratory, in both an individual and race set-up. Cycling is also a highly competitive sport where even minute time differences can affect the outcome of a race. For example, analysis of the finishing times of time trials for the 2004 Athens Olympics show that the difference between first and second was just 0.52% (Currell and Jeukendrup, 2008).

3.1.1 Rationale

Given the results from Chapter Two, where men exposed to AND (a putative pheromone and component of male odour) were found to feel and behave (as judged by third party raters) less attractive, it is predicted that similar suppressing effects might occur in this study. By using a competitive context that may be more applicable to intra-sexual signalling, the effect of male odour on other men might present itself as a performance output. Specifically, it is predicted that exposure to male odour will be associated with decreased cycling performance.

In view of the recent evidence indicating that competitiveness may be exhibited in odour, having demonstrable effects on physiological responses, a comparison of the effects of contextual male odour is made (Adolph *et al*., 2010). Male odour taken from a competitive context and a neutral context was used as olfactory stimuli in this study, and their effects on performance compared to each other and to a no-odour control condition. In Adolph *et al*. (2010), they only detected an increase in SCR when participants were exposed to a "competitive odour", as opposed to a neutral one. With this in mind it is predicted that the male odour will have a graded effect on cyclist performance, with competitive odours having the most suppressive effect, followed by the neutral male odour, and then the no-odour control.

3.2 Methods

3.2.1 Participants

Eight healthy, non-smoking, male cyclists aged between 20-35 (mean±SD = 28±5.24) were recruited from cycling clubs in the Stirling area. Only individuals who undertook two or more training sessions per week of 1-5 hour duration, and who had been cycling for at least four years, were eligible to take part. An overview of subject information can be found in Table 3.1. Participants were screened for olfactory sensitivity with the Burghart "12 screening test" Sniffin' Sticks (Hummel *et al*., 1997) before completing a general health questionnaire (Appendix VIII) and a Physical Activity Readiness Questionnaire (PAR-Q) (Thomas *et al*., 1992) (Appendix IX).

Table 3.1. Participant information, including age (yrs), mean dominance score (1-5 highly dominant), weight (kg), $V0_2$ max score and their highest Wattage reached in the ramp up test (Wmax).

Male	Age	Dominance score	Weight (kg)	VO_2 max	Wmax
1	27	3.3	76.2	62.1	450
2	30	3.9	80.1	53.4	425
3	20	3.1	74.45	61.5	400
4	33	3.3	75.4	61.8	425
5	26	2.2	75.5	63.1	425
6	22	2.9	66.7	73	425
7	35	2.7	88.3	61.9	400
8	31	3.8	82.6	50.8	350

Data were collected over a five month period, such that all participants were in a similar phase of their training cycle (i.e. their winter break). At least three familiarisation trials were completed before testing began, with the exception of one participant who had two due to his

personal time constraints. The aim of the familiarisation sessions was for the cyclists to experience the work load of the session and the use of the equipment. All trials were completed within a 12 week period with at least seven days between trials, but no more than 14 days. For two cyclists, the time between trials exceeded 14 days due to the Christmas break, therefore an extra familiarisation trial was completed by each male to reduce the effects of a break in routine.

Throughout the course of the study, participants were required to keep their training schedule constant by recording physical activity in a diary. They were also required to keep their diets as similar as possible from week to week to avoid interference with metabolic assessments. Physical activity records and food diaries were inspected every week before testing commenced. All of the sessions, including the baseline session, were performed with the participants in a fasted state.

3.2.2 Ethical approval

All participants were given an information sheet before deciding to take part. They were told that the aim of the study was to investigate how odour affects male behaviour in a competitive environment, but were given no more information regarding the different odour stimuli to be used. All participants signed an informed consent sheet before participation. Participants were reimbursed £5 a session for taking part in this experiment, as well as the results of their times and their VO_2 max score. This study was approved by the University of Stirling Psychology Ethics Committee and the University of Liverpool Committee on Research Ethics.

3.2.3 Odour donors

A total of 18 male donors from Stirling University Football team donated axillary sweat for this study. Men wore cotton pads attached to their axillary region during both a competitive match (a cup final game, which they won) and during a training session. The training session matched the

physical demands of the match, yet lacked direct competition with another team or with team-mates. In total, 11 men took part in both sampling sessions, as well as four more that only competed in the match and 3 more who only took part in training, (participant information can be found in Table 3.2). Donors were instructed to refrain from consuming spicy food and alcohol, and from smoking or using any scented cosmetics during both the evening before and the day on which they wore the pads. Additionally, donors washed with non-perfumed soap immediately before sampling on each occasion to ensure the sample was representative of the match or the training session only.

Samples were then pooled according to context, homogenised, divided into small portions (0.4g approx) and placed in sealed bags stored at -20°C (Adolph *et al*., 2010).

Post sampling, donors were asked to complete an 11-item dominance questionnaire from the international personality items pool (http://ipip.ori.org/; Goldberg 1999) (Appendix IV) and to rate how competitive they felt during the training session and the match on a scale of 1-7 (1 being not very competitive at all, 7 being extremely competitive). The score of competitiveness for the training session (M= 5.07±.83) was significantly lower than the score for the game session (M= 6.43±.514) (paired t-test: ($t(10) = -5.37$, $p<.001$). When prompted throughout the study to state a difference in intensity of the two odours, cyclists did not report any difference. This suggests that the only factor different between the two experimental odours was the social context from which they were obtained.

Table 3.2. Odour donor's subject information including mean age, dominance score and competitiveness score (all ±SE) in the competitive and neutral conditions.

	Competitive context (n=14)	**Neutral context (n=15)**	***t***	**p**
Age	22.9 ±1.1	21.4 ±.35	-	-
Dominance score	3 ±0.11	2.9 ±0.09	-	-
Competitive Score	6.4 ±0.5	5.1 ±0.8	-5.42	<0.01

Note. Ratings of dominance ranged from 1-5 (1 = not dominant, 5 = highly dominant). The competitive score ranged from 1-7 (1= very little, 7= very much). P value calculated from a paired sample t-test

3.2.4 Procedure

The experiment followed a within-subject, double-blind, randomised block design. All exercise took place on the cyclists' own road bicycles attached to an indoor bicycle ergometer (CompuTrainer™). The rear wheel was inflated to 800 kPa after which the system's load generator was calibrated to a rolling resistance of between 0.88-0.93kg. This calibration process was done before and directly after a 10 minute warm up at 150W to ensure accurate calibration as recommended by Davison *et al*. (2007). The ergometer was linked to a computer so that the wattage could be adjusted via the CompuTrainer™ software. The computer was connected to an overhead projector, enabling the screen to be seen by the cyclist. A cadence magnet was taped to the crank and a sensor attached to the rear frame of the bike to measure wheel revolutions per minute (RPM); a measure of speed.

3.2.4.1 Baseline testing

In a baseline session, participants performed a VO_2max test to determine a maximum power measurement (Wmax) to be used as a guide in the trial sessions. The VO_2max test is a graded exercise procedure to induce exhaustion on the cycle ergometers (Kuipers *et al*., 1985). After a 10-minute warm up at 150W, the ramp-up test began at approximately 175W. As the wattage was increased every minute by 25W, participants were also asked how they felt on the Borg scale (Borg, 1982). This is a scale ranging from 6-20 which enables athletes to give a subjective rating of perceived exertion (RPE).

During the ramp-up test, when participants reached an RPE score of around 14 (somewhat hard/hard) they were handed a mouthpiece to use which was attached to the Douglas Bags ready to collect expired air. The bag was changed every 45-seconds to allow for change over time before the wattage was increased by the minute. When the subject reached exhaustion, the equipment was switched off and the air in the final Douglas bag was analysed immediately. The final watt stage that the subject completed before exhaustion was recorded as their Wmax. The resulting levels of CO_2 and O_2 in the Douglas bags went towards the calculation of the VO_2max score. This score is an indicator of how the participants could cope with the ensuing experimental trials. The results of the VO_2max test and other subject information can be found in Table 3.1.

3.2.4.2 Trials

In cycling, time trials are a reliable performance test as they generally have a low coefficient of variation (CV <5%) (Jeukendrup *et al*., 1996). They are also recognised to provide a valid representation of what occurs during performance (Currell and Jeukendrup, 2008). The distance covered in this time trial was 20km; this was chosen to adhere to the time constraints of the study and to allow for a preload cycle beforehand. This distance has been used previously by Palmer *et al.* (1996) who reported a CV of 1.11%; a reputable score in comparison with other time trial distance CVs (For a review see Currell and Jeukendrup, 2008). During the familiarisation (and

experimental) trials, participants first completed a 10-minute warm-up at 150W, followed by a 40-minute preload cycle and a 20km time trial.

Given the existing evidence that competitive chemosignals can cause electrophysiological changes in the recipient, it seems necessary to investigate any neuro-endrocrinological effects. One way of addressing this is to measure energy expenditure using indirect calorimetry, which in turn gives information on hormonally controlled metabolic processes. This was done by comparing the compositions of expired air with the inspired ambient air during a preload cycle each session. The resulting measurement from this process is known as the respiratory quotient, which is a ratio of carbon dioxide produced to oxygen consumed. As different energy sources require different amounts of oxygen to catabolise, the respiratory quotient can infer the type of energy source being utilised. For example, carbohydrates have an RQ around 1.00 whereas fats and proteins, which tend to be more structurally complex have RQs around 0.7 and 0.82 (Krogh *et al*., 1920).

During the 40-minute preload cycle, one minute gas samples were taken every ten minutes. This collection process could not be performed during the time trial as the participants were left alone without distraction. Gas samples were collected in Douglas bags and analysed using a Servomex 5200 gas analyser, which was calibrated to measure the percentages of CO_2 and O_2 in the sample. A Harvard Dry Gas Meter and a Variac driven Vacuum System was used to extract and measure the volume of gas from each Douglas Bag.

The workload of the preload cycle was predetermined by each of the subject's Wmax; in this case the wattage was set to 50% of their Wmax. This ensured a moderate ride, without causing too much fatigue before the subsequent time trial. After a short break, participants were left in isolation and without music to complete a 20km time trial. The screen visible to them displayed a virtual cyclist on a track and information of the distance covered, but they were unaware of their time. This was to ensure that participants did not compete with themselves throughout the course of the experiment. As well as time to completion, speed, RPM, watts and heart rates were also recorded.

The experimental trials followed the same protocol described above, but with the addition of an odour stimulus. In each experimental trial cyclists were exposed to a fresh odour sample, which was removed from the freezer at least 30-minutes prior to exposure. The allocated bag containing sample (in the form of shredded cotton pads) was emptied into a perforated tube, which in turn was attached to a head set. This allowed the odour to be positioned in front of the nose, in a constant and hands-free manner (Fig. 3.1).

It was hypothesised that competitive chemosignals may have greater influence during a competitive context; therefore in the final two sessions, cyclists were paired according to ability level and asked to race during the time trials. Pairs were selected by matching past times from previous familiarisation trials and experimental sessions. During the race, animations of both cyclists were visible on the screen so that each could see where they were on the course in relation to their opponent (Fig. 3.2).

Following completion of their physical exercise trials, participants were asked to complete an 11 item questionnaire on dominance from the International Personality Items Pool (http://ipip.ori.org/; (Goldberg, 1999) (Appendix IV).

Figure 3.1 Subject wearing the headpiece during a trial

Figure 3.2. A pair of participants racing, during a competitive time trial

3.2.5 Analysis

Analyses were carried out using IBM SPSS Statistics 20 and separated in terms of time trial and metabolic measures. Mauchly's test was used to assess the equality of variances of the differences between conditions. Where sphericity was violated, Greenhouse-Geisser correction was used. To test for differences between conditions during the time trials, a repeated measures ANOVA was used with odour as a within-subject factor (competitive, neutral and control taken from familiarisation-trial two) and time trial result as the within-subject measure. This analysis was repeated taking into account the subject's dominance score (high or low) as a between-subject factor to test whether baseline trait dominance influenced how participants responded to signals of competition.

A second analysis was performed, this time with the RPM values taken from the time trial. The RPM max scores and RPM averages were used as within subject measures in the same analysis as above. The remaining measures of the time trial (i.e. peak power and speed as well as average power and speed) were included in a third analysis.

All of the analyses testing the effect of the three odour conditions (competitive, neutral and control) were performed on data from the individual trials, as opposed to race trials. This is due to the use of the familiarization trial data for the control condition, which participants completed alone.

To test the interaction between odour type and context of the trial, a repeated measures ANOVA was performed with odour type (competitive, neutral) and session context (individual, race) as within subject factors and time trial result as a within subject measure.

Calculated from the RQ, a respiratory exchange ratio (RER) was used in which the analysis was performed. Like the RQ, the RER is a measure of CO_2 produced to O_2 consumed, yet only during more intensive exercise. In such cases, additional factors such as hyperventilation cause the pulmonary exchange of O_2 and CO_2 to no longer reflect just the substrate mixture of energy metabolism alone. Four bags were analysed from each preload cycle, giving 4 RER

values from each session. For the analyses, RER values were averaged and compared across conditions. To account for the possibility of RER fluctuations within the trial, bags 1-4 were analysed independently with respect to odour condition.

3.3 Results

3.3.1 The effect of condition on finishing times – time trials

As expected, there was a significant main effect of context on finishing times; with race sessions producing faster times than individual sessions (F (1, 7) = 131.682, p <.001). However, there was no significant interaction between odour type and context of the trial for time trial results (F (1, 7) = .079, p = .79).

Male odour was found to reduce time trial results. Although there was not a significant effect of odour type by itself (F (2, 14) = 2.95, p = .085), planned contrasts revealed that there was an overall effect of male odour compared to the control (control vs. competitive and neutral condition, (F (1,7) = 17.84, p = .004) (Fig. 3.3). There was no significant interaction between trait dominance and odour type for time trial results (F (2,5) = .032, p = .969).

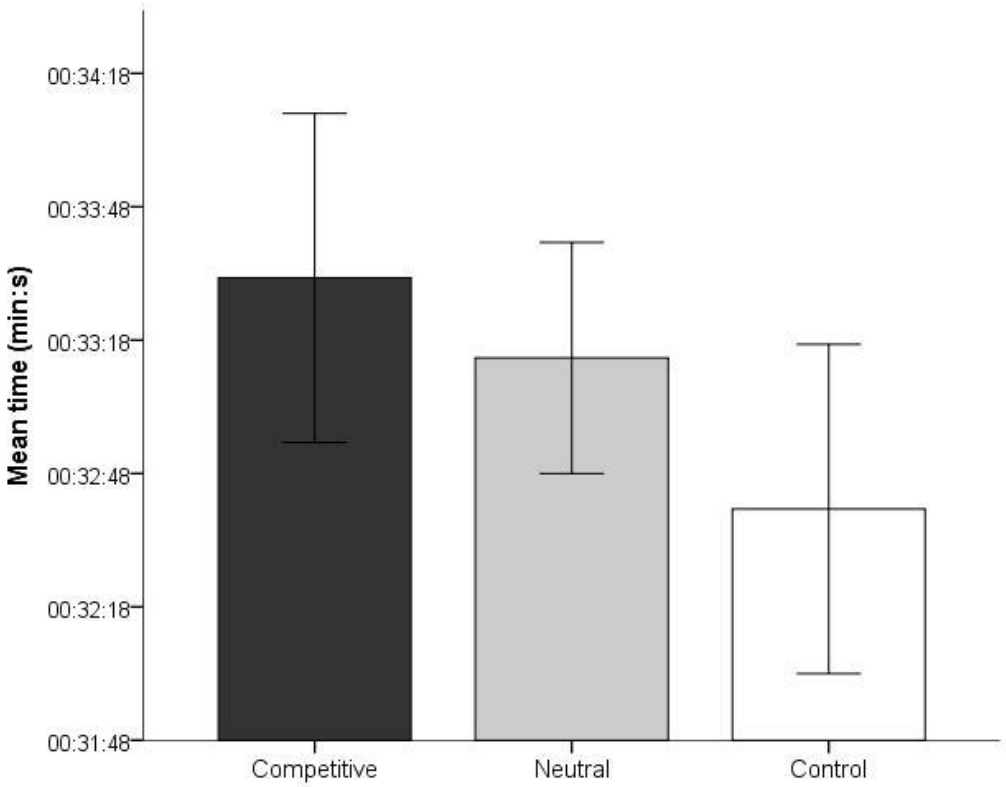


Figure 3.3. Mean finishing times for individually completed time trials, across odour conditions. (M ± SEM) Comparison of odour sessions vs. control sessions, p = .004

3.3.2 The effect of condition on revolutions per minute (RPM)

Consistent with time trial results, odour was also found to affect RPM scores, specifically RPM max (F (2, 14) = 6.047, p = .013). Planned contrasts suggest this significance is driven by the difference between odour and control sessions as with the time trial scores (control vs. competitive and neutral condition, (F (1, 7) = 8.972, p = .02)) (Fig. 3.4). Interestingly, there was a tendency for the RPM max scores to be slightly higher in the competitive odour condition than the neutral, however this difference is not significant (Paired samples t-test, t (7) = 1.182, p = .276, r = .86). There was no effect of odour on average RPM scores (Greenhouse-Geisser corrected: F (1.063, 7.443) = 1.687, p = .235).

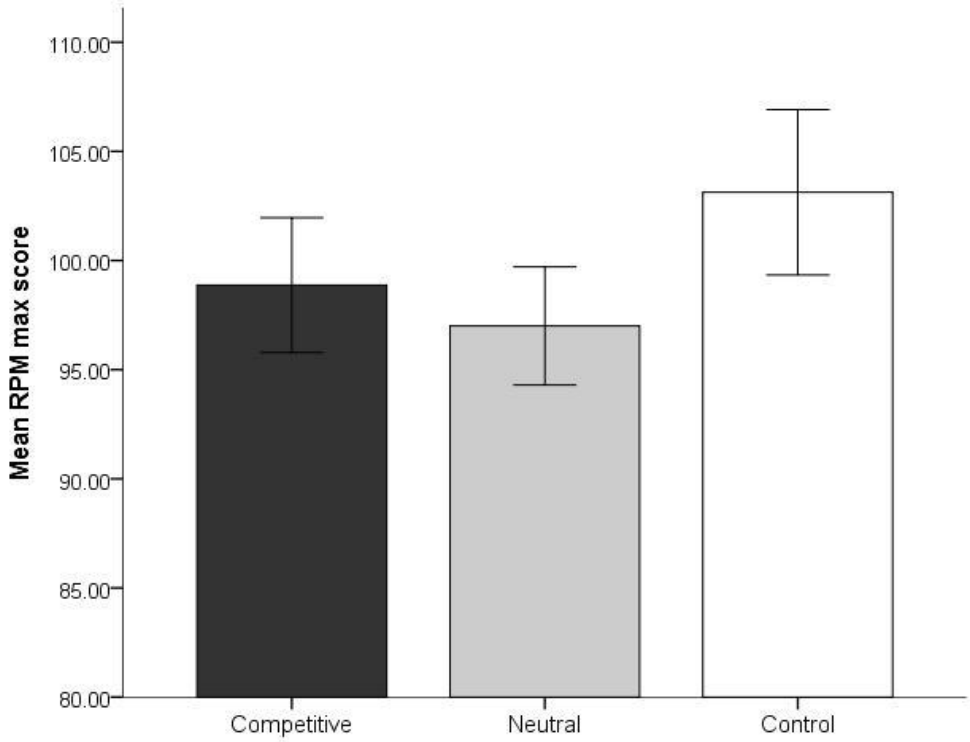


Figure 3.4. Mean (±SEM) RPM max scores from individually completed time trials, across odour conditions. (Comparison of odour sessions vs. control sessions, p = .02)

3.3.3 The effect of condition on wattage and speed

Analysis of the remaining time trial data revealed no significant main effect of odour type on peak/ average watts (p = 0.756 and p = 0.065 respectively) nor peak/ average speed (p values are 0.953 and 0.069 respectively). Yet planned contrasts showed that there was an overall effect of odour on average watts (F (1, 7) = 15.18, p = .006) and average speed (F (1, 7) = 17.52, p = .004). With lower power and speed scores in the odour conditions, compared to the control.

3.3.4 The effect of condition on metabolic measures

Average respiratory exchange ratios (RERs) were calculated for each session and can be found in Table 3.3. There was no effect of odour type on averaged RER values across individual trials (F (2,14) = .820, p = .461) or on RER values from racing trials (two odour types being neutral and competitive) (F (1,7) = .035, p = .858). Trait dominance score had no effect on odour influence (F (2, 12) = .245, p = .786). There was also no effect of odour type on RER values per bag (F (8, 24) = .788, p = .618).

Table 3.3 Mean RER values (±SE for each odour condition, along with ANOVA results comparing conditions for each bag.

	Odour			**ANOVA**	
	Competitive	**Neutral**	**None**	**F(2,14)**	**P**
Bag 1	0.94 (±.021)	0.93 (±.019)	0.95 (±.020)	1.08	0.36
Bag 2	0.93 (±.023)	0.92 (±.018)	0.92 (±.027)	0.28	0.76
Bag 3	0.92 (±.017)	0.90 (±.026)	0.94 (±.026)	2.24	0.15
Bag 4	0.91 (±.023)	0.90 (±.022)	0.92 (±.022)	0.48	0.63

3.4 Discussion

This experiment aimed to test the effect of competitive chemosignals on cycling performance in males. Following recent evidence that has shown competitive chemosignals to have a stimulatory effect on human electrophysiology, it was hypothesised that they might also influence behaviour in the appropriate context. The results suggest that in general, male odour has a negative effect on performance, as measured by a cycling time trial. Exposure to both neutral and competitive odour samples during a time trial resulted in significantly longer time scores than in the no-odour control condition.

3.4.1 Competitive vs. neutral odour

The difference in the effect of the two odour contexts (competitive or neutral) was not quite significant. This particular finding contrasts somewhat with Adolph *et al*. (2010), whose findings showed significant differences in skin conductance readings between the two odours, however the difference between general odour conditions found is encouraging. It was hypothesised that male odour in general would be associated with decreased performance, and the competitive odour would have the most powerful effect. Although there were some hints of a graded effect of contextual odour, as discussed below, there was no significant difference in measures between the two odour conditions. This disparity could be due to the fact that the sampling surroundings for the neutral odour condition contained an element of social competition. This odour sample was taken from the donors during a group training session, so despite scores of explicit competitiveness differing, the players may have still been experiencing more competitive feelings than they would in a solitary exercise control.

3.4.2 The effect of male odour on speed

The RPM max scores were found to be significantly lower in both of the odour conditions compared to the control, a result consistent with the reduced time trial scores. However, further investigation suggested that there was a tendency for scores in the competitive condition to be higher than the neutral condition. This difference was not significant, however, and there was no effect on average RPM throughout the trial. Future studies could assess this difference, as this may provide an explanation for the overall slower time trial score; could it be that the cyclists "burnt out" too soon with a high RPM max value in response to the competitive signal? This would require knowledge of when the peak RPM value occurred during the trial, which was not available in this study. It does however raise the question: is there a time limit on a competitive signal? Bearing in mind an experimental trial could last up to two hours, would we be more likely to see a difference in performance between odour conditions with a more short-term exercise task?

It may have been the case that by measuring performance based on endurance abilities as seen in this study, we are missing the opportunity to assess a "fight-or-flight" response to competitive signals that has been identified in animals (e.g. Müller-Schwarze *et al*., 1984). This is addressed in the study in Chapter Four.

3.4.3 The influence of context

It was hypothesised that competitive chemosignals may only be relevant in matching competitive contexts. Therefore two competitive trials were added to the study design, where participants would race against an opponent. As expected, time trial results were generally improved when participants had a partner to race against. However there was no interaction between odour type and session context, implying that odour had the same effect in both individual and race sessions.

The context from which the odour samples were taken should also be considered when reviewing these results. In this case, body odour extracts were taken from a team of footballers during a competitive game and a neutral training session. As stated previously, the presence of team mates in the neutral context may have still initiated feelings of competitiveness causing the odour samples to be more similar than intended. Additionally, it should be noted that football is a team sport that requires communication, trust and camaraderie to ensure maximum performance. In contrast, the cycling involved in this study was a solitary task that relied on self-awareness and concentration to succeed. It would therefore be interesting to extend this study with odour samples taken from individual sportsmen such as cycling or tennis.

3.4.4 Trait dominance interactions

There was no interaction between odour condition and trait dominance score. This could potentially be due to the small variation of dominance scores amongst the cyclists. It was hypothesised that less dominant males might react differently to the odour stimulus than more

highly scoring dominant males; yet with no real divergence within the range of scores, the lack of an interaction effect is expected. Further work with a larger sample size or different ability levels may be worthwhile.

3.5 Final conclusions

In general, the findings may imply that male intra-sexual odour signals have a suppressive effect on the perceiver. This claim is consistent with animal literature, where dominant male chemosignalling can have a suppressive effect on surrounding male competitors. For example, the urinary pheromones from dominant male marsupial sugar glider (*Petaurus breviceps;* Marsupialia Petauridae) will suppress sexual activity in surrounding subordinate males by causing a reduction of their circulating testosterone levels (Stoddart *et al*., 1994).

To my knowledge, the effect of whole male odour on other men's behaviour has not been addressed previously. Yet results of studies examining the effect of putative male pheromones (AND, androstenone, and androstenol) can provide a necessary comparison. Consistent with the findings with AND from Chapter Two, androstenone and androstenol have also been reported to have a negative effect on male self-perception (1985; Filsinger *et al*., 1984). In addition, Jacob *et al*. (2001a) and Lundström *et al*. (2003a) found that AND had a negative effect on men's mood when the experimenter was a man. These latter findings might be of particular relevance to this study, where the presence of a male experimenter might have elicited conditions of male competitiveness. It could be suggested then, that the invariable nature of these results do not only support the findings from this study, but also the continued use of androstenes in such experiments. The decision to use the whole body odour sample, and not synthetic androstene mixtures, was based partly on a growing uncertainty of androstene occurrence in human odour (due to quantification studies being based on very small and unrepeated samples, as discussed in Section Three). Furthermore, to include a difference in odour contexts (competitive vs. neutral), more specific knowledge of the compounds involved would have been essential.

However, it must be noted that present findings could indicate a simple malodour effect resulting in slower time trials. That is to say, the men could have been responding to a general unpleasant odour which may have distracted and caused them to slow down. Yet as hedonic odour ratings were not collected, this cannot be certain. Furthermore, the results are consistent with those reported in Chapter Two, where androstadienone was found to depress mood, self-perceived attractiveness ratings in males and perceptions of male attractive behaviour made by third party raters. Therefore it could be suggested that these results add to the growing evidence of male intrasexual signalling in humans.

To summarize, the results from this experiment suggest that male odour has a negative effect on cycling performance, as measured by a 20km time trial. However further work is needed to investigate the mechanism behind this effect, to determine whether it is the result of an endocrinological change or simply a malodour or distraction effect. Finally, there may also be the possibility that short-term effects of the odours are being overlooked, and that the endurance test used in this study might not be appropriate to pick up a "fight-or-flight" response to competitive signals that can be seen in some animals. Nonetheless, the results are consistent with the previous chapter which indicates a suppressive effect of male odour components on male behaviour.

Chapter Four: The effect of male odour on physical performance: anaerobic capacity and strength.

4.1 Introduction

In the previous chapter I examined the effect of male odour on performance in endurance athletic contests. It was found that performance (measured by a cycling time trial) was decreased in conditions where male odour exposure occurred. In this study, the focus changes to examine the effect of male odour on short term performance and physical strength, as measured by a maximum effort cycle test.

Although Chapter Three found a significant decrease in performance with exposure to male odour, there was no significant difference between the effect of the different types of odour: competitive and neutral. Based on the findings from Adolph *et al*. (2010), where skin conductance readings were higher with exposure to competitive odour compared to neutral odour, it was thought that a graded effect of odour type on performance might emerge. Specifically, I hypothesised that competitive odours would have the most negative effect on time trial performance, followed by neutral odours, and the no odour control (which is the baseline measure). Even though there was a tendency for time trial results to be lowest in the competitive odour condition, the difference in results was not quite significant.

There was also a non-significant trend for RPMx scores (a measure of peak speed) to be higher in the competitive odour condition, despite the overall tendency for a slower trial in this condition. It was not possible to identify the point in the trial where speed peaked, although it

could be speculated that a reaction to competitive odours occurred nearer the beginning of the session, potentially causing participants to "burn out" too soon and perform worse overall. It therefore seems necessary to test this idea, comparing the effect of competitive and neutral odours in a shorter experiment where immediate reactions can be assessed.

In experiments examining the electro-physical responses to chemosensory anxiety signals (2009; 2010; 2004; Pause *et al*., 2003; Prehn-Kristensen *et al*., 2009) and competitive chemosignals (Adolph *et al*., 2010), measurements were taken simultaneously with odour exposure via an olfactometer. This enabled the collection of immediate responses that might be occurring to potentially critical odour cues. Across taxa, it is shown that sensitivity to alarm signals can be evolutionarily advantageous as they will warn the receiver, allowing them to adjust their behaviour accordingly to increase ontogenetic survival (for a review of pheromones in mammals, see Doty, 2003). These behaviour adjustments can include preparatory measures such as the increased vigilance in the black-tailed deer (*Odocoileus hemionus columbianus*) (Müller-Schwarze *et al*., 1984) or defensive immobility seen in the rat (*Rattus norvegious*)(Mackay-Sim and Laing, 1981).

4.1.1 Rationale

This study aims to investigate the effect of competitive and neutral male odours on performance in men and women. Performance in this case was gauged by a measure of anaerobic power, produced from the Wingate anaerobic test (WAnT), a 30-second maximum effort cycle test that assesses the immediate energy system (Bar-Or, 1987). It is widely used to measure muscle power, muscle endurance and fatigability by offering a variety of measures in a short space of time (see Fig 4.1).

By using a shorter experimental protocol, which involves short bursts of maximum effort exercise, it was hoped that the difference in effect of the two odour types might be clearer. After all, the "fight or flight" response to alarm pheromones in animals is based on a quick response by the receiver (Wyatt, 2003).

With men's self-rated attractiveness and behaviour ratings decreasing with AND exposure in Chapter Two, and a decreasing performance with male odour exposure found in Chapter Three, it is expected that similar suppressing effects in males might occur in this study. Specifically, it is hypothesised that males' performance scores will be lower with competitive odour exposure, compared to neutral odour.

This study also examines the effect of male odour on women, as well as men. It is well established that females will eavesdrop on male intrasexual chemosignalling to ascertain information on quality (Gosling and Roberts 2001). Therefore it is hypothesised that female performance may be higher in the competitive odour condition, based on evidence that competitive odours induce higher electro-physical responses than neutral odours (Adolph *et al.* 2010).

4.2 Methods

4.2.1 Participants

Forty-one individuals aged between 18 and 43 (males, n=21 mean age ± SD = 24.5 ± 6.52; females, n= 20, mean age ± SD = 23.6 ± 6.19) were recruited from the Stirling University student and staff population. All participants were healthy, non-smokers, and with no current injuries or olfactory disorders. Prior to acceptance on the study, participants were required to complete an online medical history questionnaire that highlighted any injuries and assessed current activity levels (Appendix VIII). Only those in good health, who did not suffer from any present injuries and who met the NHS physical activity guideline for adults (150 minutes of moderate intensity aerobic activity every week) were able to take part in this experiment. Following completion of their physical exercise trials, participants were asked to complete an 11-item questionnaire assessing trait dominance from the International Personality Items Pool (http://ipip.ori.org/; (Goldberg, 1999) (Appendix IV).

As in Chapter Three, participants were informed that the aim of the study was to investigate how odour affects behaviour in a competitive environment, but were given no more information regarding the odour stimuli that would be used.

4.2.2 Ethical approval

All participants were given an information sheet before deciding to take part. They were told that the aim of the study was to investigate how odour affects sporting performance, but were given no more information regarding the different odour stimuli to be used. All participants signed an informed consent sheet before participation. Participants were reimbursed £5 a session for taking part in this experiment, as well as the scores from their session. This study was approved by the University of Stirling Psychology Ethics Committee and the University of Liverpool Committee on Research Ethics.

4.2.3 Odour stimuli

Odour stimuli were provided from the sampling session used for Chapter Three, with 18 male donors from Stirling University Football team. Men wore cotton pads attached to their axillary region during both a competitive match (a cup final game, which they won) and during a training session. The training session matched the physical demands of the match, yet lacked direct competition with another team or with team-mates. Participant information can be found in Table 3.2 (Chapter Three, section 3.2.3).

As before, samples were pooled according to context (neutral/ competitive), homogenised, divided into small portions (0.4g approx), and placed in sealed bags stored at -20°C (Adolph *et al*., 2010).

4.2.4 Experimental design and procedure

4.2.4.1 The wingate anaerobic test (WAnT)

The Wingate anaerobic test (WAnT) is a 30-second maximum effort cycle test that assesses the immediate energy system. It is widely used to measure muscle power, muscle endurance and fatigability by offering a variety of measures in a short space of time. It has been shown to be sensitive and repeatable, indicating that the scores will reflect a person's actual performance rather than a random occurrence that might change from one measurement to another (Bar-Or, 1987). The test is also non-invasive and not athlete specific; it can be practiced on participants who do not need any special skills or training using equipment commonly available (Bar-Or, 1987). Furthermore, the results of the WAnT are likely to be influenced by self-motivation and testing environment (Wilmore, 1968), which in this case was manipulated through exposure to different odours.

There are a range of scores produced from the WAnT, which are detailed points A-F in the example test in Figure 4.1. There is a measurement of peak power (PP) (point A) which is the highest wattage reached usually in the first five seconds of the test. Anaerobic fatigue (point B) can be calculated as a percentage decline in power throughout the test, representing the sustainability of the immediate energy system. Total anaerobic work, an overall measurement of energy expenditure, can also be calculated. With a wide ranging population sample, power scores can be made relative to body mass (Wkg^{-1}) to allow direct comparisons between individuals. Speed is measured in terms of revolutions per minute (RPM), as seen in Chapter Three. Maximum speed is referred to as RPMx (point C). Average power and minimum power (point D) scores are also recorded as well as various time measurements, including time to peak power (tPP) (point E) and time to RPM max (tRPMx) (point F).

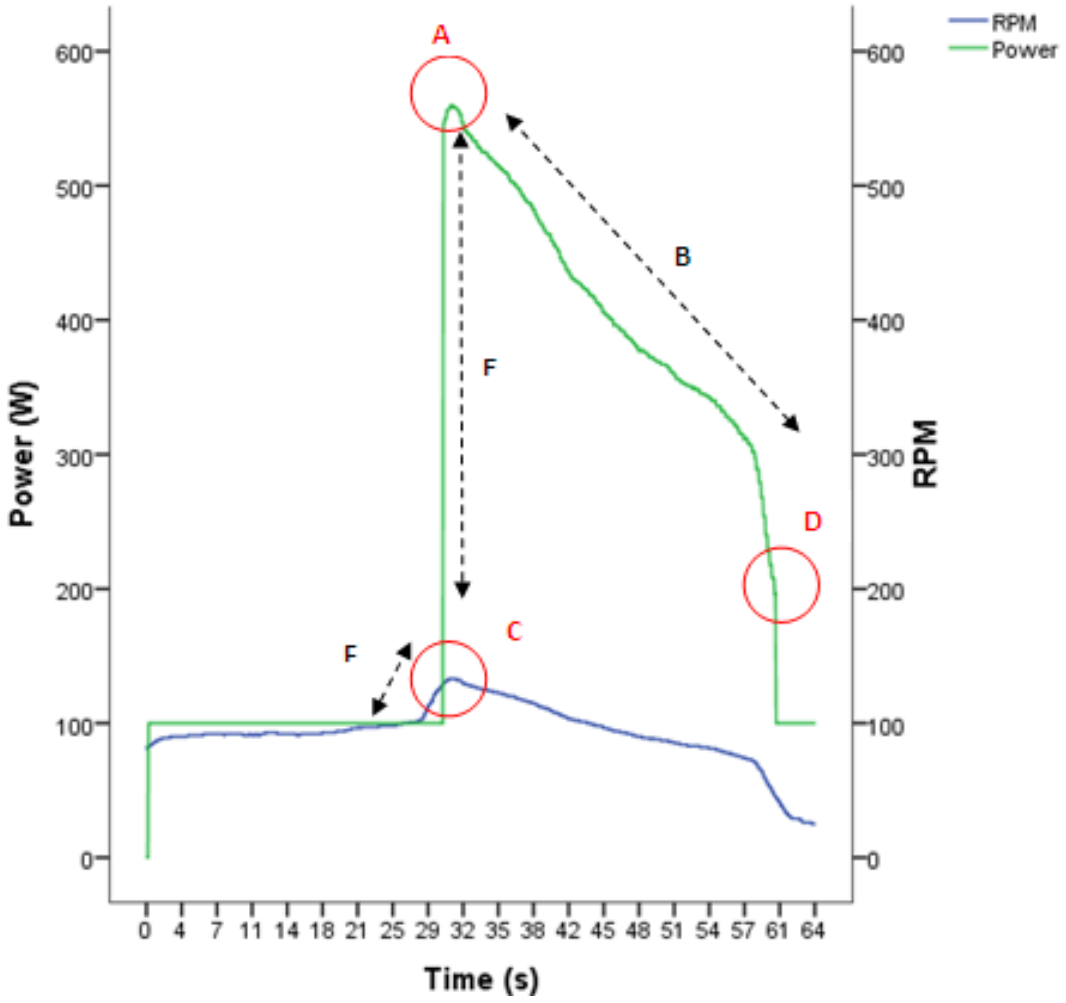


Figure 4.1 A graphical representation of the results from an example Wingate test. Points A-F highlight the different scores achieved at each point in the test, as detailed in section 4.2.4.1.

4.2.4.2 Procedure

Each participant attended two sessions, one for each odour condition (competitive and neutral). The WAnT took place on an electromagnetically braked Lode Excalibur Sport cycle ergometer with the flywheel resistance equal to 0.075 kg per kg of body mass according to the optimization tables of Bar-Or (1987). Since previous research has revealed that exercise performance varies with time of day (Baxter and Reilly, 1983; Reilly, 1987), the two sessions were held at

approximately the same time. It is unknown whether climate has an effect on anaerobic performance in adults (Dotan and Baror, 1980), so a room temperature of 20°C was maintained with an air conditioning system.

Upon arrival at the testing sessions, participants were weighed and their height was recorded. To avoid the intra-individual effect of postural changes on cycling performance (Dorel *et al*., 2009; Peveler *et al*., 2007), foot position on the pedals, saddle height, and upper body position of each subject were recorded and replicated in the second session. Toe clips were used to prevent the participants' feet from slipping during the test. During the experimental condition, the odour stimulus was presented using the same device described and used in the study reported in Chapter Three.

To begin the exercise, participants were asked to complete a 10-minute warm-up on the cycling ergometer at a self-selected wattage, with 5-second intermittent sprints every three minutes as recommended by Inbar and Bar-Or (1975). To prevent over-pedalling during the sprints, the resistance was increased momentarily, typically 50% higher than their chosen resistance, to allow for the increased effort. The self-selected resistance and sprint resistances were recorded so that the same procedure could be followed in the second session. After a brief pause, the wattage was set at 100W and participants were asked to begin pedalling so that the test would start from a rolling resistance. This build-up period lasted 1-minute and made up the final minute of the 10-minute warm-up.

Participants were alerted to the fact that the resistance would seem difficult during the test and that it was essential for them to try as hard as possible for the full 30-seconds. Participants were given a countdown from 5-seconds to when the main resistance would be applied, and were encouraged to start maximum effort within the last 2-seconds of starting, in order to gain momentum. They were given verbal encouragement and a verbal countdown in the last 10-seconds by the same experimenter each time; the experimenter was blind to the odour condition. A 5-minute cool down proceeded at the subject's own selected resistance, which was again recorded

so that this resistance could be used in the second session. After a 10-minute break, a second WAnT was performed using the protocol described above in order to give an indication of recovery.

The data from the two tests were recorded using Lode Ergometry Manager™ software, a programme that adjusts the resistance according to body weight and records each data point of the test, every 0.5-seconds. The warm-up was programmed into the ergometer manually, without use of this software, to allow for the intermittent sprints and for the participant to adjust their resistance.

4.2.5 Analysis

For each test, peak power, mean power, and cadence (RPM) scores were recorded. A fatigue index was calculated with the Lode Ergometry Manager™ software using the following equation:

Anaerobic fatigue = (peak power – minimum power) ÷ peak power x 100

Analyses were conducted using IBM SPSS Statistics 20. In all comparisons, the data from the first test in the session was used, unless stated otherwise. To test for differences between odour conditions, for each sex a repeated measures ANOVA was used with odour (competitive and neutral) as a within-subject factor and power score data, RPM scores, time measures and fatigue index as within-subject measures.

The results from the dominance questionnaire were used to allocate individuals into two categories: high scores and low scores. This allocation was based on the median dominance value (2.72), those with a higher score than this were classed as "high" and those with a lower score were classed as "low". The next set of analyses aimed to test whether baseline trait dominance influenced how participants responded to signals of competition. The analyses described above were repeated, but this time taking into account interactions with gender with dominance score (high or low) by including them as between-subject factors.

A final analysis included order of stimuli presentation as a between-subjects factor, to assess whether the novel testing conditions in the first session may have affected the influence of the odour. This was further investigated with a paired samples t-test to compare first and second scores of peak power, mean power, and RPM max.

4.3 Results

4.3.1 Power scores (peak power, average power, and minimum power)

There was no main effect of odour type on measures of power ($F(3, 36) = .791$, $p = .507$) or an interaction of odour type and sex ($F(3, 36) = .837$, $p = .483$). The same applies for results in the second test (main effect: $F(3, 33) = .721$, $p = .547$; interaction: $F(3,33) = 1.165$, $p = .338$). There was however, a significant effect of odour on female's minimum power scores (the lowest power point during the test) ($F(1,16) = 4.68$, $p = .046$), with lower power scores occurring in the competitive condition (M±SE = 145.64±8.90) compared to the neutral condition (M±SE = 161.14±9.96) (Fig. 4.2). There were no significant differences of the remaining power scores between odour conditions for females (peak power, $p = .721$; mean power, $p = .829$).

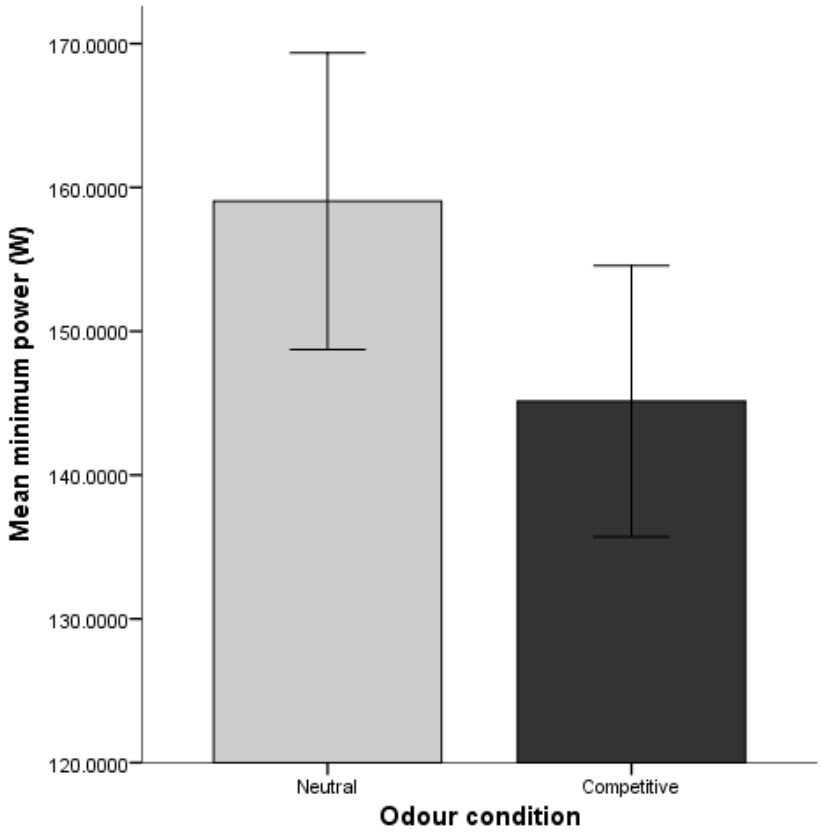


Figure 4.2. Mean minimum power scores for females between odour conditions (M±SE). There was a significant effect of odour condition on minimum power in females, p = .046.

4.3.2 Average time scores (tPP and tRPMx)

When times were averaged to include both tests one and two, there was no main effect of odour type on tPP and tRPMx ($F(3, 38) = .023$, $p = .977$) or an interaction of odour type and sex ($F(3, 38) = 1.5$, $p = .236$). Yet visual inspection of the data shows that there was a trend for males to take longer to reach peak power, and females to take less, although this result is not significant ($F(1, 34) = 3.285$, $p = .079$) (Fig. 4.3).

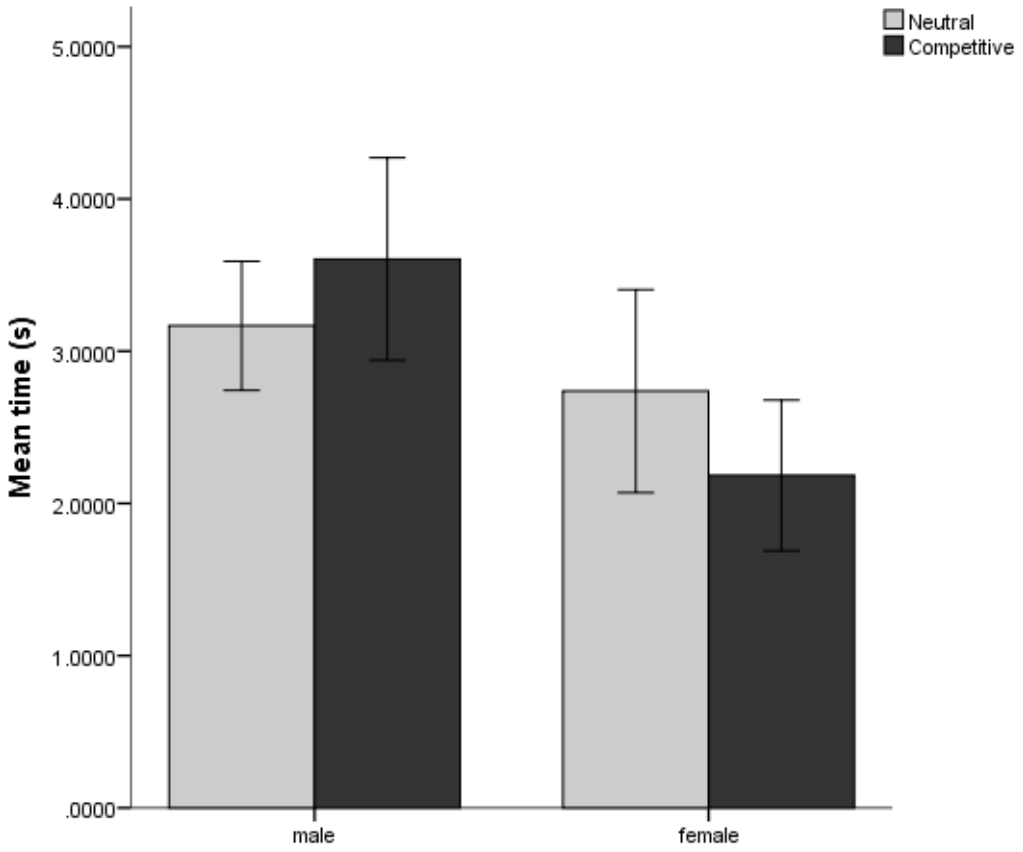


Figure 4.3 Mean time in seconds (taken from an average of tests 1 & 2), took to reach peak power (±SE) (p = .079).

4.3.3 Effect of odour condition on rate of fatigue

The data reveal that female fatigue measures are significantly different between odour types during the second test in the session (F (2, 14) = 3.84, p = .047). This is driven by a near significant difference in rate of fatigue scores between odour groups (paired sample t-test: t (15) = -2.093, p = .054) where the rate of fatigue was higher in the competitive odour condition (M±SE = 70.41± 2.20) than in the neutral odour condition (M±SE = 67.84 ± 2.53).

4.3.5 Investigating the interaction with participant dominance scores

There was no interaction between trait dominance score, odour condition and sex (F (7,21) = .386, p = .9) or between trait dominance and odour condition itself (F (7, 21) = .737, p = .644).

4.3.6 Investigating the interaction with stimuli presentation order

There was a significant interaction between order of stimulus presentation and odour type for averaged values of all within-subject measures from both session tests (F (7, 31) = 2.591, p = .032). This was driven by three measures which showed an interaction between order and odour type: peak power (F (1, 37) = 8.524, p = .006), mean power (F (1, 37) = 4.12, p = .05) and RPM max (F (1, 37) = 8.058, p = .007). A comparison of the first and second session scores alone, without taking into account odour, shows that first session scores of peak power, mean power, and RPM max were significantly lower in the first session (Table 4.1).

Table 1.1 Mean scores, with standard deviation, for peak power, mean power and RPM max for sessions one and two. Results of paired t-tests are given for comparisons across the two sessions.

	1st Session		2nd Session		t - Test	
	M	**SD**	**M**	**SD**	**t**	**P**
Peak power	687.27	208.25	711.91	222.37	-2.967	.005
Mean power	540.26	161.63	546.20	160.89	-2.084	.044
RPM max	135.01	20.88	139.46	21.83	-2.86	.007

4.4 Discussion

The aim of this study was to test the effect of male competitive chemosignals on the anaerobic capacity and strength of men and women, as measured by the Wingate anaerobic test. This type of short term, maximal effort test was chosen to assess the possibility of a "fight or flight" response to competitive chemosignals. Odour type seemed to affect certain aspects of the WAnT results in a sex-dependent manner, which is discussed below.

The amount of time a subject takes to reach their maximum power (tPP) could represent their psychological state. It could be speculated that if they felt enthused and motivated to try their best, then they might reach the target power in less time than if the they were distracted in some way. It is interesting then that odour type appeared to have an effect on the tPP in a sex-dependent manner, following visual inspection of the data (although the result was ultimately not quite significant). When exposed to the neutral odour, both males and females took a similar time to reach peak power. However, when exposed to the competitive odour sample, females tended to take less time and males took more. For females, this result was reinforced by a near-significant increase in rate of fatigue and a significantly lower minimum power during competitive odour exposure.

Taken together, these findings could suggest that male competitive chemosignals have a potentially stimulatory effect on females, as measured in the WAnT. This could be seen as consistent with sexual signalling theories, whereby females will be more responsive to male odours cueing high dominance and genetic quality (Moore, 1997). This has been shown widely across all taxa (for a review see Johansson and Jones, 2007). Havlicek *et al*. (2005) demonstrated this to some degree in humans, revealing that women prefer the odour of more dominant males during the fertile phase of their menstrual cycle. It is possible that the competitive odour used in this study may not directly translate to a dominant signal as specified by Havlicek *et al*. (2005), yet similarities can be drawn given the assumption that competing (and winning) will increase testosterone levels (Archer, 1991; Booth *et al*., 1989; Christiansen, 1998), a physiological trait

associated with dominance (Archer, 1991; Christiansen, 1998). Furthermore, the increase in males' tPP may reinforce the findings from Chapter Three, which indicated that male odour could have a suppressive effect on other males.

It is well established that females will eavesdrop on male intrasexual chemosignalling to ascertain information on quality (Gosling and Roberts 2001). In this case, the male competitive chemosignals may have subconsciously initiated female interest, causing the stimulatory response that is suggested by the results. This would also be consistent with Adolph *et al.* (2010), who reported that females, as well as males, exhibited a higher skin conductance response when exposed to competitive odours compared to a control. For this to be investigated further, some measure of female hedonic valence or other arousal measures might be necessary. In this study however, hedonic ratings were not taken in order to keep the participants from focussing on what the smell might be. Practice trials indicated that attention on the odour throughout the study reduced concentration and in some cases may have initiated an aversion response that would not have occurred had the odour been omitted from the focus of the study.

Odour effects did not seem to be dependent on trait dominance, consistent with the findings from Chapter Three; nor was there an influence of odour on the main test scores of peak power and mean power. This may be explained by the interaction effect found between order and odour type that is driven by first session scores being consistently lower than second session scores. Therefore the influence of different odours may have been limited by the novelty of the first session, where inexperience of maximal exercise leads to slightly lower test scores.

4.5 Final conclusion

In summary, there is some indication that male competitive chemosignals may be influencing neuromuscular activity and immediate energy availability, or individual motivation. For females, competitive male odours appear to be having a stimulatory effect during the WAnT, perhaps causing them to reach their peak power in less time but potentially feel increased fatigue as a

result, leading to lower minimum power scores. Males on the other hand take longer to reach their maximum power, this may be indicative of a suppressive effect of competitive chemosignals on male performance as suggested in Chapter Three. Overall scores of absolute power appear to be uninfluenced by odour type. Yet it could be suggested that this may be due to the lack of a practice session; results illustrate that the first trial produced consistently lower scores, possibly because the participants were novel to the testing procedure.

Male human odour as an intra-sexual signal: general discussion

The experiments in Chapters Three and Four present novel evidence for an effect of male odour on physical performance. This is shown in two contexts: long-term endurance athletic contests and short-term, immediate muscle strength responses. In each case, male odour exposure was found to have a negative effect on men's performance in some way. Following previous findings showing a difference in competitive and neutral odours, contextual male odour effects were also compared in these experiments. Results indicate that competitive chemosignals have a potentially stimulatory effect on women; this could be seen as consistent with sexual signalling theories, whereby females will be more responsive to male odours cueing high dominance and genetic quality (Moore, 1997).

The findings presented in this section support the idea that male intrasexual signalling may be occurring in humans, paving the way for further investigation into a context that has not yet been so directly tested. This does not mean that these findings discount the possibility of a role of odour in human mate choice – both functions could be important in the same signal, and evidence for an effect on female behaviour includes the possible stimulatory response of females to competitive odours in Chapter Four (and those shown in Adolph *et al.* 2010). Indeed, it may well be the case that women effectively eavesdrop on male-male odour signals to ascertain information on quality, as seen in animals (e.g. Gosling and Roberts, 2001).

General similarities of these results can be drawn to the work from Sell *et al.* (2010; 2009) who has reported that humans can accurately predict the upper body strength of males from just facial cues (Sell *et al*., 2009) and auditory information (Sell *et al*., 2010). Sell *et al.* argue that in order to make accurate assessments of interpersonal risk, humans will have adaptive mechanisms

in place to assess potential cues of fighting ability. It would be interesting to see if this ability exists multi-modally in humans, where odour cues might be used to make competitor assessments. Although my experiments do not explicitly address the assessment of strength through odour, preliminary conclusions may be drawn in view of the behavioural results from this study. That is to say, males may have subconsciously perceived a 'threat' in the competitive odours, causing the trends of decreased performance. This idea would merit more investigation, perhaps with an explicit test of odours from strong and weaker men.

In Chapter Three, it was speculated that the decrease in performance with exposure to male odour (competitive *and* neutral) may have simply been a result of a malodour effect, whereby the presence of a potentially unpleasant odour could have had negative consequences on performance. If this was the case, we might not expect to see the sex-dependent response of male odour seen in Chapter Four. Although these results are not directly comparable, as there was not a no-odour control in the study in Chapter Four, the stimulatory response in females would not be expected if male odour was simply acting as a malodour depressant.

In summary, these findings are novel in two respects. First, the sex-dependent responses to male odour provide evidence of both intra- and inter- sexual odour signalling in humans during a competitive context. Second, they are the first to provide empirical evidence of a behavioural response to chemosensory cues of emotional state, where previous studies have focussed mostly on physiological changes. However, the conclusions drawn from these results are based on various assumptions about measures of power (for example, tPP as an indication of effort; an idea that has not yet been directly tested), therefore it is clear that there is scope for more research. Finally, although the use of composite male odour has been found to be an adequate and relevant stimulus to use, future work would benefit from knowing the specific compounds that elicit such sex-specific effects. This is addressed in Section Three.

SECTION THREE: ANALYTICAL ASSESSMENT OF HUMAN AXILLARY ODOUR – AN EVOLUTIONARY APPROACH

The final section of this thesis attempts to bridge the gap between odour chemistry and social behaviour in humans. Using analytical chemistry techniques alongside human behaviour assessments, the studies in this section neatly assess androstene content in sweat as well as their effect on perceptions of attractiveness.

A large part of this thesis has been dedicated to understanding the role of the 16-androstenes in humans. Originally thought to be act as sex pheromones on account of their role in pigs (Signoret and du Mesnil du Buisson, 1961a), studies over the last 60 years have focussed on their effects in a mate choice context. The majority of these studies, without exception of those in this thesis so far, have focussed on the effect of a single androstene compound. The choice of compound and the concentration in which it is presented seem to be, for the most part, arbitrary. As Table I illustrates, early studies favoured the use of androstenol and androstenone whereas more recent investigations focus on the effect of androstadienone. The reason for this shift in interest is unclear and perhaps a cause for concern in androstene research; androstadienone may not be the best androstene representative as it has been reported to be a likely precursor of the more odorous androstenol and androstenone (Brooksbank *et al*., 1972; Nixon *et al*., 1988; Rennie *et al*., 1991). Furthermore, many androstadienone studies use the unrealistically high concentration of 250µM, as used originally by Jacob and McClintock (2000) but who gave no explanation for using this concentration. Previous studies that date back 60 years ago have given some indication of

androstene concentrations in human axillary sweat (see Table II), yet results are variable and in some cases based on small numbers without repeat sampling.

The interest in human pheromone research continues to develop. Furthermore, various novel findings that use whole body odour samples, rather than a single and perhaps questionable compound, are increasingly reported. Is it time to re-evaluate our understanding of axillary odour content? Certainly, more knowledge on androstene abundance would be beneficial, yet perhaps a more constructive aim would be to link their abundance to aspects of quality.

For the 16-androstenes to be truly considered as human pheromones, we would expect a number of things:

1. That their occurrence in odour would vary between individuals.
2. Variation between individuals will correlate with indices of quality.
3. Exposure to them in the correct concentration and context would elicit a response in another individual. Furthermore, it is predicted that any potential pheromone compounds to be uncovered will be acting in a complex mixture rather than on their own.

Chapter Five tests the first two conditions with an analytical assessment of male and female odour. The results from this were then compared to several aspects of phenotypic quality and socially relevant traits such as attractiveness, masculinity and relationship status. The aim of Chapter Six is to test the third condition: the elicitation of a behavioural response. Using a synthesised mixture of compounds based on the results of Chapter Six, the effects of two valid androstene mixtures on attractiveness

Table II. A summary of studies that quantify the 16-androstenes in axillary sweat

Study	Sample size* / repeat sampling	Sampling technique	Method	Brief summary of findings
(Brooksbank *et al*., 1974)	12 M / Pads worn for a period of 5-7 days	Pads worn and extracted using acetone and methanol and a tracer quantity of androstadienone	GC/MS	AL and probably AN, do occur in male axillary sweat. The inter and intra individual variation in concentration is large; giving support to their possible function of human pheromones.
(Labows *et al*., 1979)	7M / No	Adrenalin injection and collection of sweat with micropipettes.	(Direct injection of secretion into the chromatograph) GC/MS	Although compounds were found at the same retention times as AN and AL, these specific compounds were not detected as such from the fresh aprocrine sweat, suggesting that they are formed in the axillae by the action of enzymes or bacteria.
(Bird and Gower, 1982)	6M/ 6 collections made from each axillae, at least 24 hrs interval	Gauze pads were worn by participants. Hair not shaved.	Radioammunoassay	Significant difference in androstenone levels between axillae, between individuals and within individuals over time. Use of antibacterial material reduced amounts of androstenone but not Squaline or cholesterol, suggesting that androstenone is produced by metabolism of a precursor in the axillae by skin-micro-organisms.
(Gower *et al*., 1985)	11M 16F/ No	Axillary secretions collected from right and left armpits	Radioammunoassay	AN levels in men varied widely (5.2-1019 pmol/24h), but were mostly lower in women (1.2-16.6pmol/24h) - apart from one woman who had a concentration of 551 pmol/24h).
(Preti *et al*., 1987)	3M, 4F/ 3 times a week for 14-16 weeks	Pads worn on each axillae for 6 to 9 hrs	GC/MS	The concentration of AL varied during the collection period. Males appear to produce more AL at certain times; women's secretions show a menstrual variation in AL; the highest concentrations of this compound appear to be produced in the mid-follicular phase, prior to ovulation.

(Nixon *et al*., 1988)	28M/ No	Axillary hair cut (not shaved) and treated to extract compounds,	Combined capillary GC/MS with specific ion monitoring	First simultaneous quantification of AN, AND and AL in axillary hair. No relationship between compounds present and age. Further evidence to suggest bacterial breakdown of AND into AN and AL.
(Rennie *et al*., 1991)	7M 2F/ No	Adrenalin injection and collection of sweat with microcaps plus cup-scrubbing method to determine the presence of 16-androstenes on the skin surface	GC/MS. The 16-androstenes were derivatized to the *tert*-butyldimethylsilyl ethers.	AN found in the secretion of all subjects, contents ranging from 46 to 630ng/µl of sweat. AND found in 4 out of 7 male apocrine sweat secretions and in the women; wide range of concentrations. AL detected in 5 males 2 females. Ether skin washings showed small amounts of all androstenes, namely AND, in all subjects bar one. Evidence to suggest AND is the precursor to the other androstenes, by axillary coryneform bacteria.

Note. compound abbreviations: AN refers to androstenone, AND to androstadienone, AL to androstenol
* Sample size is explained in numbers of males and females; M = male; F = female.

*Chapter Five: Quantification of androstene steroids in male axillary odour and their links to phenotypic quality indicators.

***The work in this chapter was done in collaboration with Dr Jan Christensen (University of Copenhagen) and Prof Patrizia d'Ettorre (University of Paris 13). These collaborators conducted the GC/MS analyses described here and helped with initial analysis**

5.1 Introduction

Chapter Five describes a study which aims to link the underlying chemistry of male axillary odours to known phenotypic and psychometric indicators of quality, as well as other socially relevant traits. Individual variation in 16-androstene steroid content of axillary sweat was analysed using gas chromatography coupled with mass spectrometry (GC-MS) and the results were correlated with various phenotypic and psychometric measures. It was hypothesised that if the 16-androstenes are acting as pheromones in humans, their abundance in axillary sweat will be varied amongst individuals and be correlated with various other quality indicators.

The quantification of the 16-androstenes in human apocrine sweat has been the subject of much research over the past 50 years. Subsequent to their discovery in the pigs (Signoret and du Mesnil du Buisson, 1961a), several attempts have been made to find similar compounds in humans through the use of GC-MS analysis (see Table II, Section Three, for a summary of studies that quantify the 16-androstenes in human axillary sweat). Differences in androstene content between individuals may serve as clues to their biological role. Comparison between the sexes indicates higher levels of androstenone (Gower *et al*., 1985), 5α- androstenone (Bird and Gower, 1983) and

dehydroepiandrosterone (Preti *et al*., 1987) in men than women. This suggests that androstene expression might be sexually selected, and indeed these previous studies interpret their findings as evidence that androstenes may be acting as male pheromones to female receivers. Studies of urinary androstenol levels indicate that androstene production begins at puberty, particularly in males (Cleveland and Savard, 1964). This sexually dimorphic pattern of expression, which emerges around puberty, is also characteristic of a trait that is subject to sexual selection (e.g. see Andersson, 1986).

There is, however, some debate over the validity of studies using GC-MS to identify axillary androstene compounds, particularly for the earlier studies. In particular, previous studies tend to use small sample sizes, often of less than 10 individuals (Table II, Section Three), and the lack of repeatability over time cast doubt over the accuracy of results. A recent debate over GC-MS sampling methods has also highlighted some methodological issues, such as analysing headspaces above samples where it is thought no relevant compounds to human odour will be found. This is claimed to be because the important odour constituents are polar molecules that tend to bind to sampling materials (Preti *et al*., 2006). Yet Curran *et al*. (2005) have argued against this claim, being of the opinion that headspace molecules are still important contributors to odour and that their absence in aqueous phase sweat is not a valid reason to disregard them during the analysis. Indeed, human produced volatile compounds which can be identified in headspace, but not in sweat, have been found to play a large role in odour interactions such as mosquito attraction (Bernier *et al*., 1999).

In this study, direct analysis of the sampling materials was undertaken on the basis that androstenes are sufficiently volatile to be perceived at short distance and play a role in communication in other mammals (Signoret & du Mesnil du Buisson 1961). Design of the study was also improved over previous androstene-based analysis studies: a large number of men were sampled (n=40), and repeatability was assessed by sampling from both axillae as well as over the course of three consecutive days. Finally, a range of other measures which are putative quality indicators (e.g. body size, facial attractiveness) were collected from the same individuals in order

to test for the first time the predicted associations between androstene expression and individual or mate quality.

5.1.1 Chemical links to quality

Across a wide range of taxa, females show preferences for phenotypic traits that are thought to provide indicators of underlying genetic quality (for a review, see Andersson, 1994). If the 16-androstenes have pheromonal properties in humans, it is expected that their abundances would vary between individuals and that this variation would be correlated with other phenotypic indicator traits. They might also be expected to vary within individuals as a correlate of more fluid quality indicators such as behaviour or social status. This is tested in the current study, by comparing androstene abundance with already established indices of human quality, such as morphometric measures and other socially relevant traits which are detailed below.

5.1.1.1 Morphometric measures

Human studies have consistently reported that females will show a preference for male physical traits that may indicate increased physical strength. For example, taller men are perceived as more desirable to date (Jackson and Ervin, 1992), as well as being found to date more often (Shepperd and Strathman, 1989) and to have higher reproductive success (Nettle, 2002; Pawlowski *et al*., 2000). Men with a low waist to chest ratio are also generally found to be more attractive (Fan *et al*., 2005; Maisey *et al*., 1999). Greater male upper body strength could be interpreted as an adaptation for intrasexual competition in terms of fighting ability, yet it could also be associated with hunting and resource provision during intersexual selection.

Many of the sexual dimorphisms in body size are due to hormonal differences during development; particularly that of higher levels of testosterone in males. It is thought that testosterone may be an honest indicator of quality in males as high levels can impose an

immunocompetence handicap (Grossman, 1985; Hamilton and Zuk, 1982; Zahavi, 1975). Testosterone-induced sexual dimorphisms develop early in humans, as measured for example by the ratio of the second to fourth digits (known as 2D:4D ratio), which is thought to be a putative marker of prenatal exposure to testosterone (Fink *et al*., 2004; but see Koehler *et al*., 2004). Given that androstene compounds and testosterone are both androgen derived steroids, it seems potentially instructive to correlate androstene abundance with morphological traits associated with testosterone.

5.1.1.2 Symmetry

Fluctuating asymmetry (FA) is the deviation from perfect bilateral symmetry caused predominantly by environmental stress and genetic problems during development (Møller and Thornhill, 1997). FA is inversely correlated with various good gene indicators such as facial attractiveness (Gangestad *et al*., 1994; Penton-Voak *et al*., 2001), facial masculinity (Gangestad and Thornhill, 2003; Scheib *et al*., 1999) and skin health (Jones *et al*., 2004).

Interestingly, FA has also found to be linked to male body odour. Rikowski and Grammer (1999) reported that for males, body FA was inversely correlated with body odour attractiveness. This was only found when the female odour raters were in the most fertile phase of their menstrual cycle. The chemical basis of this "scent of symmetry" is unknown, and to our knowledge, has not been addressed in research to date.

5.1.1.3 Facial attractiveness

Male facial attractiveness has been correlated with other phenotypic indicators of quality such as male voice attractiveness (Saxton *et al*., 2006), body shape (Hughes *et al*., 2004), and ratings of social dominance (Puts *et al*., 2006). Body odour attractiveness ratings are also concordant with facial attractiveness ratings from the same males (Rikowski and Grammer, 1999; Thornhill *et al*.,

2003). Objective attractiveness ratings of face and odour are therefore assessed during this study and compared to the chemical analysis, as well as a self-rating measure of attractiveness by the donors themselves.

5.1.1.4 Social status and personality measures

There is a growing body of research focussing on body odour changes in response to social situations, possibly cueing changes in emotions and personality characteristics. For example, emotional states such as fear and aggression can be perceived through odour (Ackerl *et al*., 2002) as well as mood states of anxiety (2009; Pause *et al*., 2004). Furthermore, the odour of more dominant males has been found to be preferred by women during the fertile phase of their menstrual cycle (Havlicek *et al*., 2005).

Androgen steroids are thought to be derived from the metabolic breakdown of testosterone (1986a, 1987; Nixon *et al*., 1984, 1986b; Rennie *et al*., 1989). Circulating testosterone levels and content of body odour will fluctuate depending on time of day (Resko and Eiknes, 1966) or as a result of social environmental factors such as shifts in status or contexts (e.g. Mehta and Josephs, 2006; Schultheiss *et al*., 1999). For example, higher testosterone levels have been associated with offensive behaviour in male judo competitors (Salvador *et al*., 1999) and tennis players after winning a match (Booth *et al*., 1989; Mazur and Lamb, 1980). In non-human primates, higher testosterone levels have been recorded when males achieve high status, and these decrease when status is lost (Eberhardt *et al*., 1980; McGuire *et al*., 1986; Setchell *et al*., 2008). There have also been reports of reduced testosterone levels in males at the beginning of romantic relationships (Marazziti (Gray, 2003; Marazziti and Canale, 2004). Given the link between testosterone levels and androstene abundance, it is expected that variation of odour content will be correlated with factors influencing testosterone levels in men.

5.1.2 Rationale

Using current analytical chemistry techniques, this study aims to link the underlying chemistry of human odour to known phenotypic and psychological aspects of quality described above. It is hypothesised that if the 16-androstenes are acting as pheromones in humans, their abundance in axillary sweat will be varied amongst individuals and be correlated with various quality indicators and/or socially relevant factors.

5.2 Methods

5.2.1 Participants

We collected axillary sweat from 41 males (mean age ± SD = 22.02 ± 2.88). Each participant came into the lab in the morning on three consecutive days, having showered with a non-perfumed soap provided the night before. Subjects refrained from using deodorant for three days prior to sampling and during the course of the study. They were also instructed not to eat various strong flavoured foods that can affect odour, such as garlic and onions. Men shaved their armpits prior to testing in order to control for variation in body hair and to facilitate swabbing of the axillary skin surface.

To obtain a general body odour sample for pleasantness ratings, subjects wore a white cotton T-shirt whilst sleeping (2 nights) that had been washed in non-perfumed detergent. The T-shirt was kept in a sealed bag during the day and returned to the lab on the last day of sampling. The T-shirts, were stored at -80°C until testing. Freezing of the samples has no effect on body odour quality (Lenochova *et al*., 2009)

In the final day of sampling, subjects completed a questionnaire assessing various personality traits previously found to have a link with odour, such as dominance and extraversion (Havlicek *et al*., 2005; Sorokowska *et al*., 2012) (Appendix X). The questionnaire also assessed other factors thought to affect odour such as exercise, diet, stress levels, hygiene, alcohol drug and

tobacco use. Various morphometric measurements were also taken from the subjects, including weight, height, size of laryngeal prominence, and 2D:4D ratio. Finally, they posed for a neutral photograph looking straight at the camera in front of a white background.

5.2.2 Ethical Approval

All participants were given an information sheet before deciding to take part. They were told that the aim of the study was to investigate the chemistry of human odour. All participants signed an informed consent sheet before participation. Participants were reimbursed £20 for taking part in this experiment. This study was approved by the University's Biological Sciences Human Ethics Review Board.

5.2.3 Sweat sampling

Samples were taken using 2.5cm^2 sections of sterile gauze (Propax, 8ply, absorbent sterile cotton gauze, BSN medical Ltd) which had been pre-cleaned with methanol during ultrasonication to drive off impurities. A standardised area of the axillae (approximately 5cm diameter) was determined using a filter-paper template overlaid on the axillary surface. Swabbing duration and technique was also standardised across participants; three drops of ethanol (≥99.5% pure, Sigma-Aldrich ®) were added to the skin and swabbed for 30-seconds with the gauze, using sterilised tongs. This process was regulated with a stop watch and repeated three times for each axilla. There were four swabbers in total, who were rotated between participants to reduce the chance that they would swab the same participant more than once. Immediately after swabbing, the gauze samples were placed in labelled 10ml amber glass vials with PTFE/silicone septa (purchased from Microlab Aarhus, DK) and placed in a -80°C freezer.

5.2.4 Chemicals for steroid extraction and analysis

Dichloromethane (Rathburn, Walkerburn, Scotland) and HPLC-grade methanol (Rathburn, Walkerburn, Scotland) were used in the extraction of the gauze and for preparation of stock solutions. All glassware was rinsed thoroughly in ethanol after washing to remove organic residues. Stock solutions of androstenol (5α-Androst-16-en-3α-ol, ≥98% purity, Sigma Aldrich, CAS=1153-51-1, MW=274), androstenone (5α-Androst-16-en-3-one, ≥98% purity, Sigma Aldrich, CAS=18339-16-7, MW=272.43), and androstadienone (androste-4,16-dien-3-one, ≥98% purity, Steraloids.com, CAS=794-58-9, MW=270.41) of 100 µg/ml were prepared in methanol. A surrogate standard solution of anthracene (CIL, Cambridge, UK) of 1 mg/mL was prepared in methanol.

5.2.5 Extraction and GC-MS analysis

5.2.5.1 Pre-treatment of the gauze pads and extraction

Post sampling, the gauze samples were ultrasonicated for 10 min in 5 mL methanol and the extract was transferred to a 30 mL amber glass vial. The procedure was repeated three times in total and the extract combined. The extract was then evaporated to dryness under nitrogen. The extract should only be dry for a few minutes to avoid extensive evaporation of the steroids. When half of the methanol had evaporated, the glass was shaken softly to ensure that the steroids did not stick to the upper part of the glass vial. The glass vials were then kept in a water bath at 40 °C during the evaporation to avoid condensation of water in the glass vials. The dried extract was then re-dissolved in 500 µL dichloromethane. The re-dissolved extract was cold-filtrated at -20 °C through a glass filter (Ahlstrom, Grade 131, 2 cm diameter), transferred to 4 mL amber glass vials, and kept at -20 °C until analysis.

5.2.5.2 GC-MS analysis

The concentrations of androstenol, androstenone, androstadienone isomers were analysed using a gas chromatograph (Agilent 6890N) interfaced to an HP-5975B quadrupole mass spectrometer (MS) operating in electron ionization (EI) mode (Agilent 5975B). The gas chromatograph was equipped with a 60 m ZB-5 capillary column (Phenomenex, Værløse, DK) with dimensions (0.25 mm inner diameter × 0.25 µm film thickness). Helium was used as the carrier gas, and the gas flow was 1.1 mL min^{-1}. Aliquots of 1 µL were injected in splitless mode. Injector, ion source, and quadrupole temperatures were 280, 230, and 150 °C, respectively. The oven program was: 100 °C (held for 3 min), increased to 200 °C (15 °C min^{-1}), and then to 310 °C with the rate of 5 °C min-1 (held for 13.33 min) leading to a total analysis time of 45 min. Selected ion monitoring was used to analyze 4 m/z values (m/z 178, Anthracene, surrogate standard), androstenol isomers (m/z 274), androstenone isomers (m/z 272), and androstadienone isomers (m/z 270). Six-point internal calibration curves of androstenol, androstenone, and androstadienone in the concentration range 0.06 – 6 µg mL^{-1} were used for quantification of the related isomers. Anthracene was used as internal standard.

5.2.6 Rating of the body odour samples from the T-shirts

Thawed T-shirts were divided into four equal sections by being cut into two (throat to navel) and then each half being cut down the shoulder (front and rear). Individual male samples were then created for perceptual testing by pairing one left and one right quarter sample because odour quality can vary according to handedness (Ferdenzi *et al*., 2009). Samples were placed into clean glass jars and sealed with aluminium foil across the lid. Samples were grouped into batches of 6-12 for assessment. There were 212 odour raters aged between 18-40 (men, n = 82, mean age (± SE) = 27.7 ± .94; women, n = 130, mean age (± SE) = 26.9 ± .59); each rater sniffed each jar and gave a rating of pleasantness, intensity and desirability using the same scale and method as Wedekind *et al*. (1995b) and Roberts *et al.* (2008). Raters were members of the public passing through the

World Museum in Liverpool, who took part on a voluntary basis, and they provided written informed consent before taking part in the ratings.

The same people also rated photographs of the odour donors for attractiveness and masculinity, using a 7-point Likert-type scale.

5.2.7 Statistical analysis

All statistical analyses were carried out using IBM SPSS Statistics 20. For visualisation of the data for this thesis, a principal component analysis (PCA) was performed with varimax rotation to identify groups or clusters of variables; in this case, logged compound abundances. Eight compound abundances were entered with a molecular mass of ~270, which resembled the 16-androstenes. Extraction of variables was based on their Eigenvalue being greater than 1. To investigate the association between androstene expression and relationship status, cluster analysis was conducted to assign individuals to groups of men with similar androstene profiles. A chi squared test was carried out to visualise the link between relationship status and cluster membership.

Compound concentrations were normalised against the total ion current (TIC), which is the sum of all the peaks in the whole chromatogram. In other words, for each compound, it is the abundance of that compound expressed as a proportion of all the compound abundances in the sample.

Odour ratings of the T-shirts were then compared in multivariate ANOVA with cluster membership as a fixed factor and log-transformed odour ratings as dependent variables. Ratings from both men and women were used, including ratings of intensity and pleasantness as well as an extra judgement of desirability in the case of female raters. Odour ratings were also correlated with morphometric measures, personality scores and attractiveness ratings using Spearman's rank correlation.

Correlations between actual compound abundances and the questionnaire results were also assessed using Spearman's Rank correlations. All questionnaire measures, including self-rated physical attractiveness, masculinity (and others included in Table 6.1) were correlated with three different expressions of log-transformed compound abundances; 1. a ratio of –enol and –enone compounds to –dienones, 2. sum of –dienones and 3. sum of –enone/–enols. Similar correlations were made for the morphometric measures taken, including height, weight, BMI and 2D:4D.

5.3 Results

5.3.1 Variability in 16-androstene expression

GC-MS analysis of axillary extracts uncovered eight compounds with a molecular mass of ~270, which appear to be androgen steroids. Although there is variability in their content across individuals (Fig. 5.1), within-individual expression of these compounds in left and right axillae are highly correlated ($r>0.95$ in each individual).

Principal components analysis (PCA) was used to reduce the number of variables. The GC-MS results were considered appropriate for principal components analysis, as the Kaiser-Meyer-Olkin measure of sampling adequacy was .624 and Bartlett's test of sphericity was significant, indicating that variables were inter-correlated (χ^2 (28) = 998.06, p <.01). Initial eigenvalues indicated that the first two components explained 73.9% and 16.7% of the variance respectively. The remaining six components had eigenvalues less than 1 and explained approximately 9% of the variance all together. The first two components were therefore extracted in the PCA; factor loadings for each of the eight compounds can be found in Table 5.1.

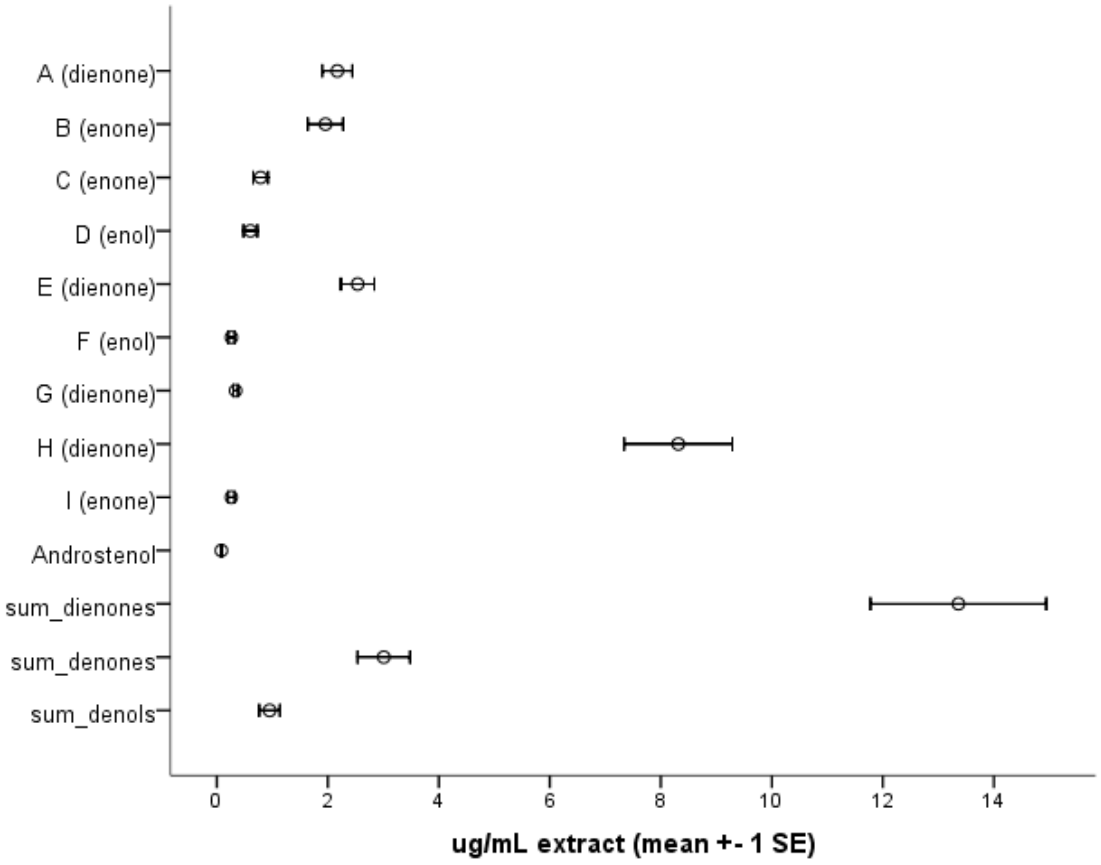


Figure 5.1. Variation in compound abundance (µg/ mL) across individuals, M±SE**.**

Table 5.1. Factor loadings from the principal components analysis (with varimax rotation) for 8 androstene-like compounds found in male axillary sweat.

	Component (cluster)	
Compound	**PC1**	**PC2**
A (*–dienone*)	0.957	0.23
B (*–enone*)	0.675	0.542
C (*–enone*)	0.654	0.542
D (*–enol*)	0.241	0.951
E (*–dienone*)	0.951	0.256
F (*–enol*)	0.204	0.949
G (*–dienone*)	0.96	0.225
H (*–dienone*)	0.951	0.249

5.3.2 Androstene expression and relationship status

Exploratory analysis revealed a potential relationship between androstene expression and the men's relationship status (i.e. whether they were single or paired). As seen in Figure 5.2, individual PC1 scores were plotted against PC2 scores for left and right axilla samples from the 41 men. Left and right samples from each individual male tend to be closely spaced (as expected from the analyses above), and the distribution of single (blue) and paired (pink) men are somewhat distinct.

To further investigate the association between androstene expression and relationship status, cluster analysis was conducted to assign individuals to groups of men with similar androstene profiles. A two-step cluster analysis revealed two groups of men, with 12 men in cluster 1 and 23 men in cluster 2. As indicated by the PCA, there was a significant association between cluster membership and relationship status ($\chi^2 = 4.143$, $p = .045$; Fig. 5.3). Eighty-three percent of the men in cluster 1 were single, and 85.7% of those in a relationship were in cluster 2. Although there is overlap in odour space between single and paired men, this seems to be further disentangled by taking into account relationship length. Figure 5.3 illustrates relationship length of paired men and it can be seen that those in relatively long relationships tend to occur in the overlap zone between single and paired men, while those in the early stages of their relationship tend to have higher PC1 and PC2 scores, based on readings from the right axillae only (Fig. 5.4).

I then compared abundance of each of the eight constituent androstene compounds in men falling into cluster 1 and 2 (Fig 5.5). Men in cluster 1 had higher abundance of each of the –enones and –enols, but lower concentration of all four –dienones (Table 5.2).

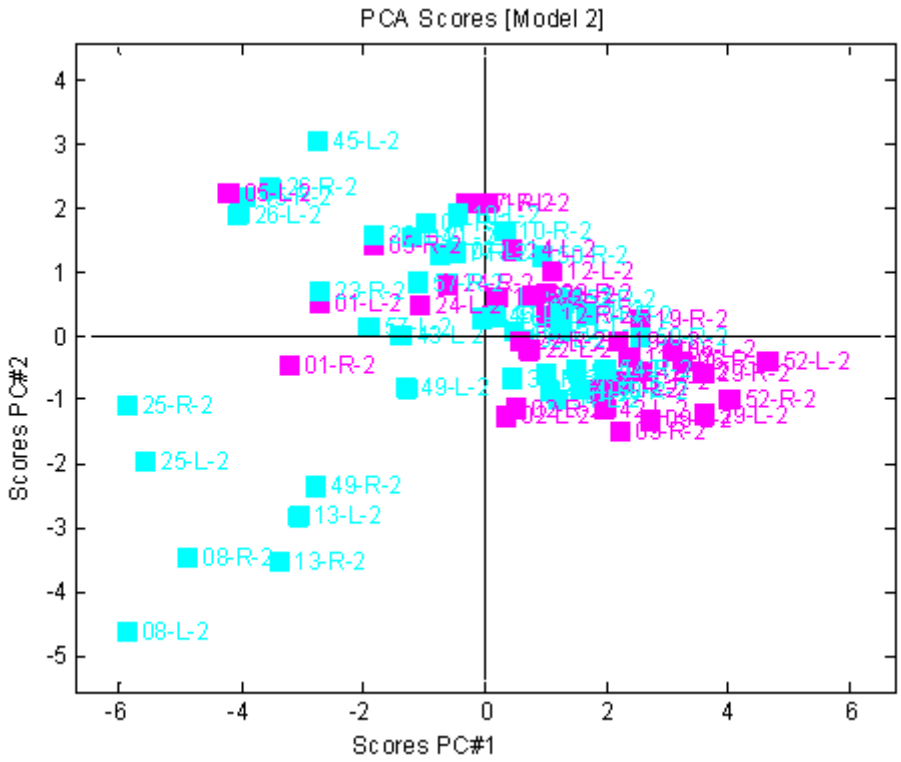


Figure 5.2 Score plot from principal components analysis indicating that androstene profiles (here displayed using PC1 and PC2) are associated with relationship status. Blue points represent single males and pink points represent those in relationships. The figure displays readings from both axillae ('R' denotes 'right' and 'L' denotes 'left'). Participant numbers are displayed alongside the point as well as sampling day. Example "13-R-2" corresponds to male 13, right axillae, day 2 of sampling.

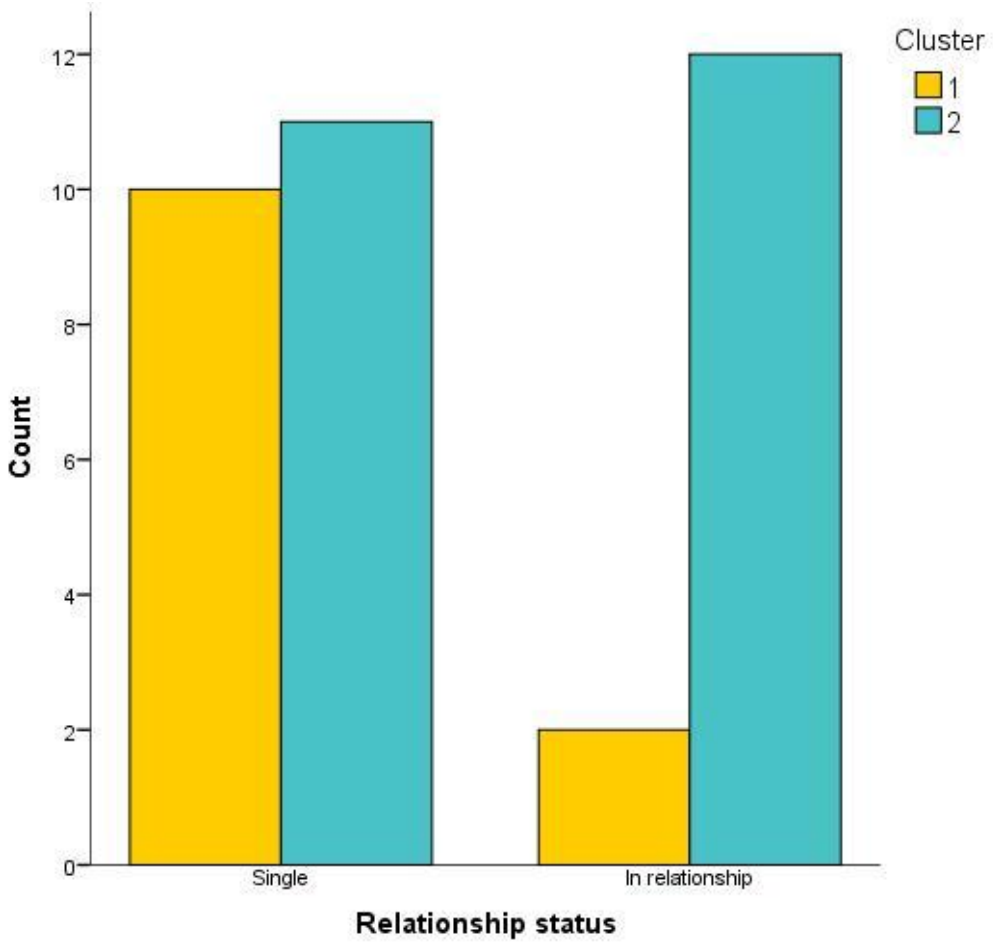


Figure 5.3 Association between cluster membership, defined by androstene profile, and relationship status ($\chi^2 = 4.143$, $p = .045$).

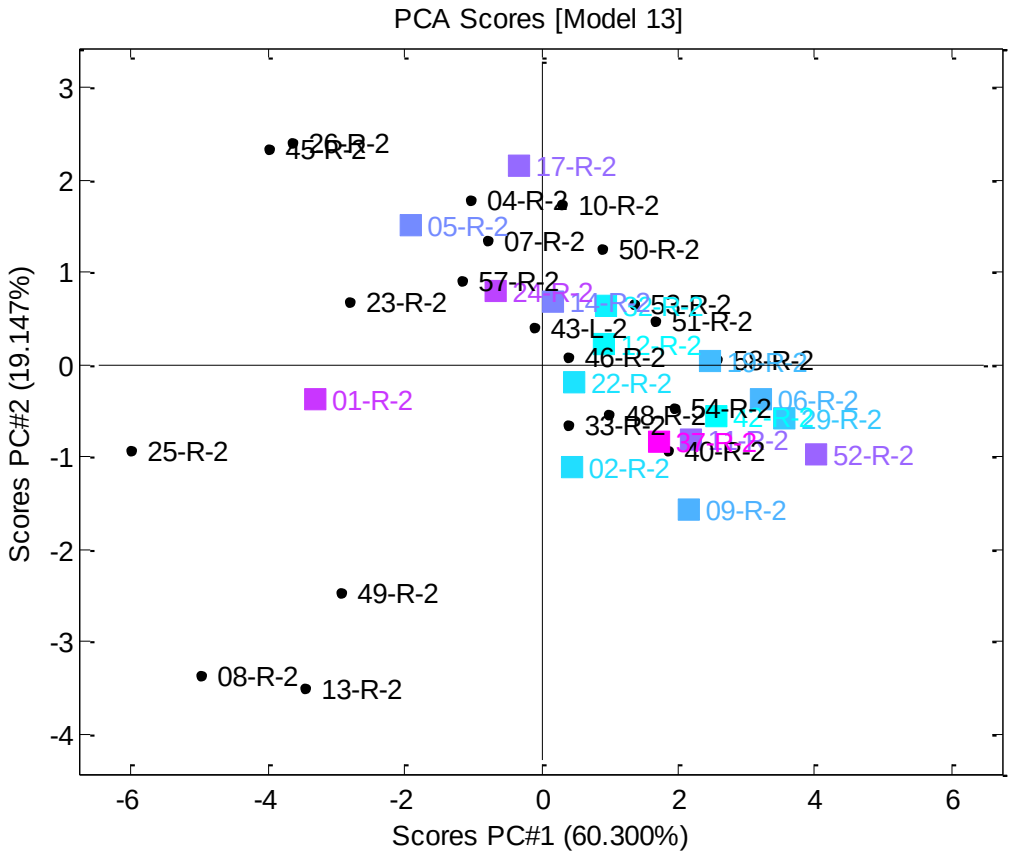
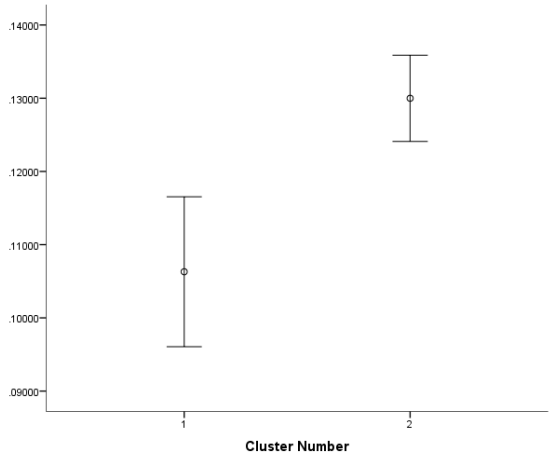
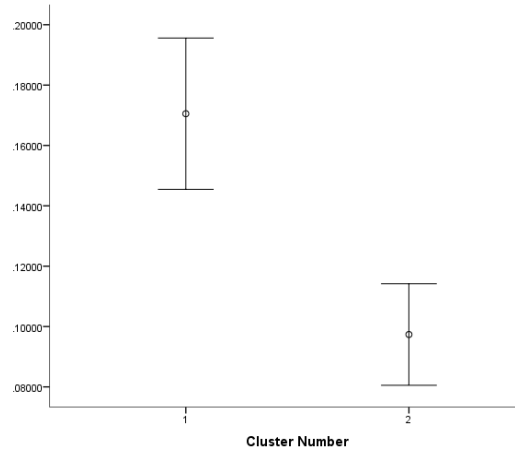


Figure 5.4 Score plot from principal components analysis. Men in relationships are colour coded according to relationship length. The more pink the point, the longer the relationship (blue represents men at the beginning of their relationships). Single men are labelled in black. For relationship length reference, male 1 = 48 months, 17 = 37 months and 24 = 45 months.

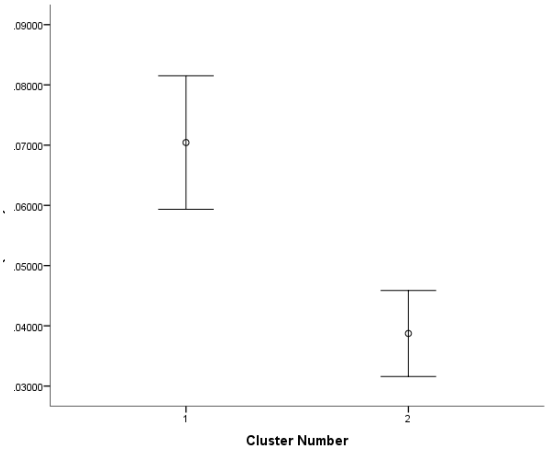
A **(*-dienone*)**



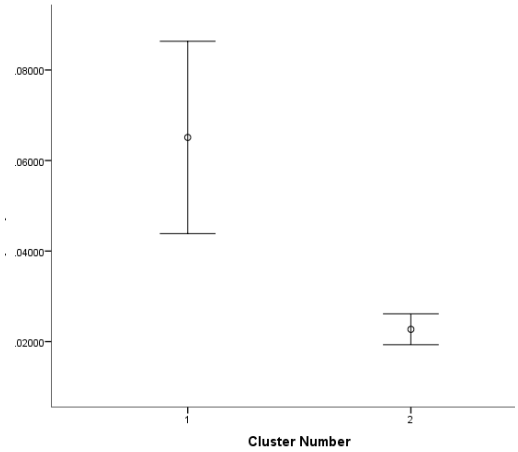
B **(*-enone*)**



C **(*-enone*)**



D **(*-enol*)**



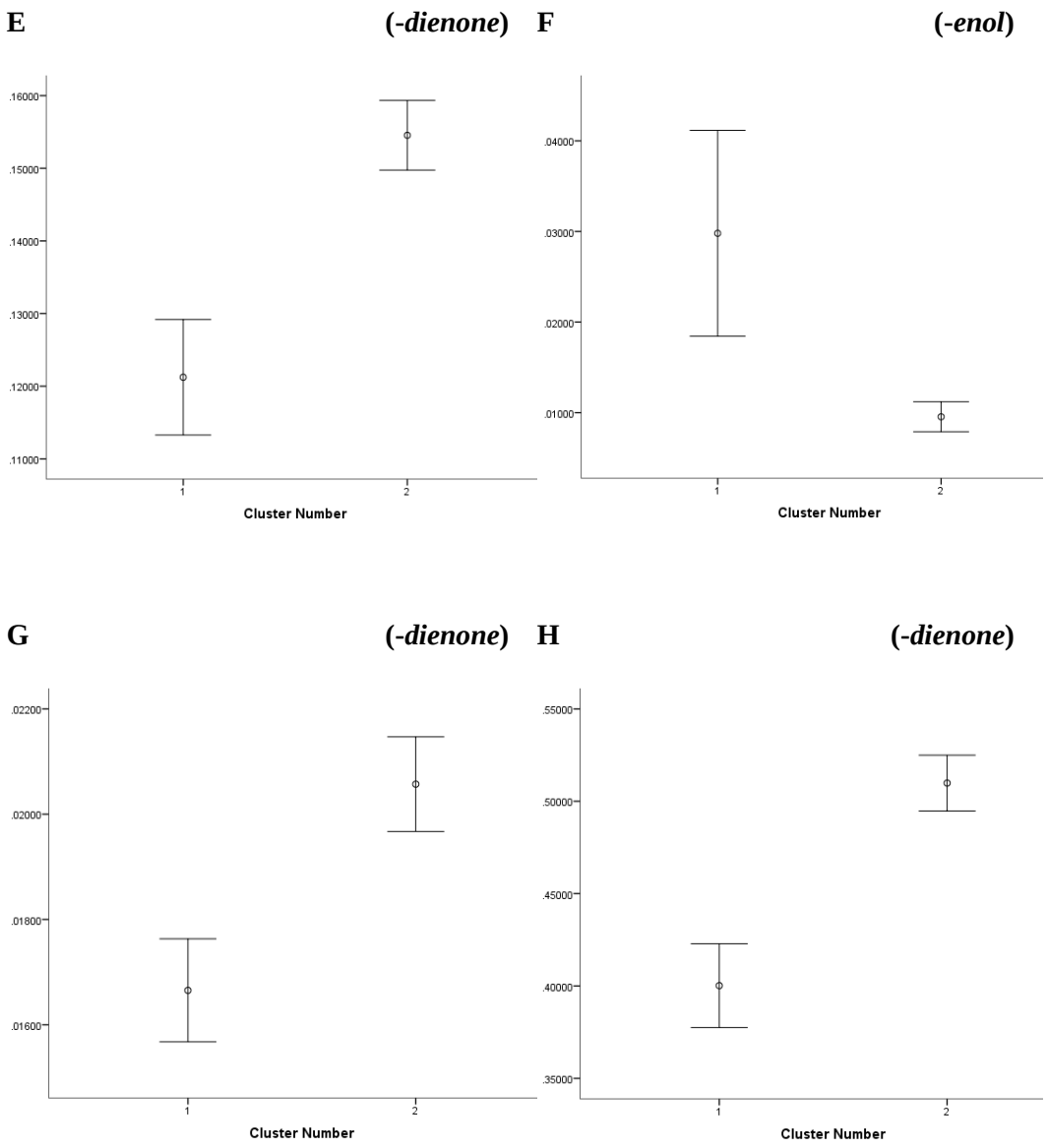


Figure 5.5. Compounds A-H and their TIC abundances in clusters 1 and 2.

Table 5.2. Comparison of compound abundance between the two clusters, as tested by an independent samples t-test.

Compound		Levene's Test for Equality of Variances		t-test		
		F	p	t	df	p
A (-dienone)		.250	.620	-4.600	33	<.001
B (-enone)		.006	.937	5.328	33	<.001
C (-enone)		.052	.822	5.344	33	<.001
D (-enol)	(Equal variances not assumed)	29.004	.000	4.288	12.685	.001
E (-dienone)		.208	.652	-8.117	33	<.001
F (-enol)	(Equal variances not assumed)	27.397	.000	3.839	12.561	.002
G (-dienone)		1.062	.310	-5.928	33	<.001
H (-dienone)		.014	.905	-8.852	33	<.001

5.3.3 Odour ratings

Independent odour ratings of the men's T-shirts were also found to be significantly associated with cluster membership (Fig. 5.6). Mean ratings of body odour pleasantness, by both male and female raters, were found to be higher for men in cluster 1 (having higher *–enones* and *–enols*) (Multivariate ANOVA, male raters: F (1,33) = 9.586, p = .004; female raters: F (1,33) = 4.305, p = .046). Female ratings of odour desirability were also higher for cluster 1 odours (Multivariate ANOVA, F (1,33) = 5.026, p = .032). There was no difference in male and female ratings of odour intensity between clusters (Multivariate ANOVA, male raters: F (1,33) = 2.938, p = .096; female raters: F (1,33) = 2.148, p = .152).

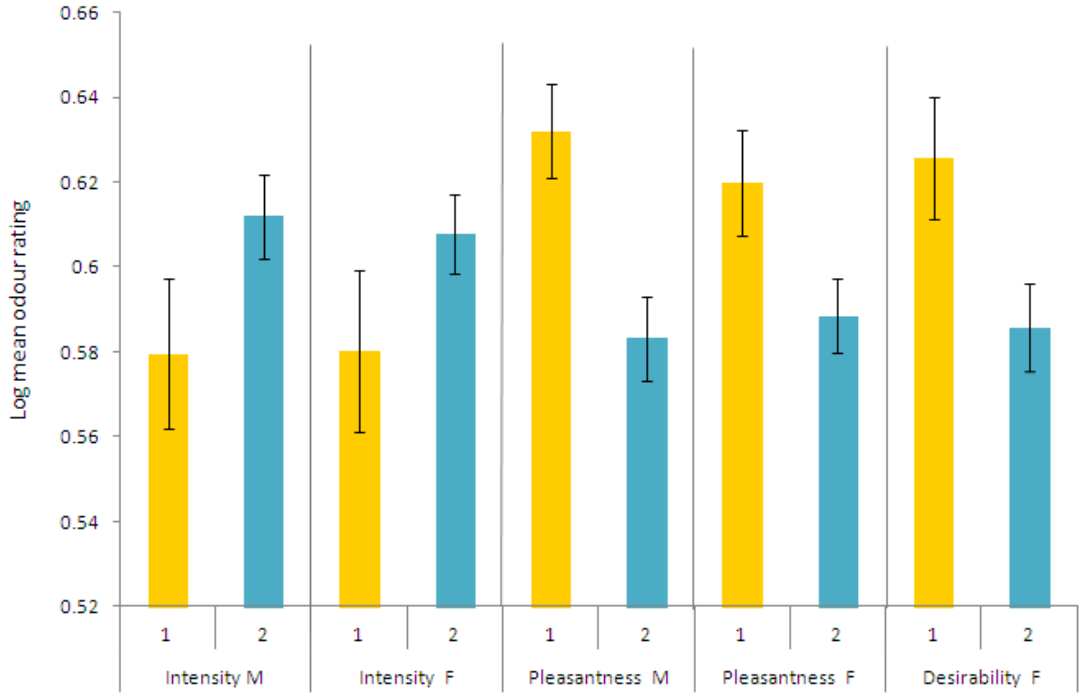


Figure 5.6 Mean ratings of T-shirts compared between clusters (M±SE). Yellow bars correspond to cluster 1; blue to cluster 2. Ratings include: Pleasantness by both males and females (high enone/enols) p = .004; female raters: p = .046); female ratings of odour desirability, p = .032; male and female ratings of odour intensity, p = .096; female raters, p = .152).

5.3.4 Other indicator traits

Compound concentrations could also be predicted by some of the self-rated personality scores. A logged ratio of *-dienone* / *-enone* and *-enol* compounds was found to negatively correlate with the men's self perceived physical attractiveness scores, as rated by a 9-item scale and a 3-scale (Spearman's rank correlations, 9-item scale: $r_s = -.376$, $p = .026$ (Fig. 5.7); 3-item scale: $r_s = -.34$, $p = .046$). These measures were therefore found to positively correlate with the logged sum of *-enone/-enol* compounds (Spearman's rank correlations, 9-item scale: $r_s = .374$, $p = .027$ (Fig. 5.8); 3-item scale: $r_s = .34$, $p = .046$). A logged measure of *-dienone* compounds was also found to negatively correlate with self-rated masculinity scores ($r_s = -.321$, $p = .049$; Fig. 5.9).

Other psychological measures, such as scores of extraversion, dominance and self-esteem as well as attractiveness scores were not predicted by androstene ratios (see Table 5.3 for a summary of the correlation results for the other measures).

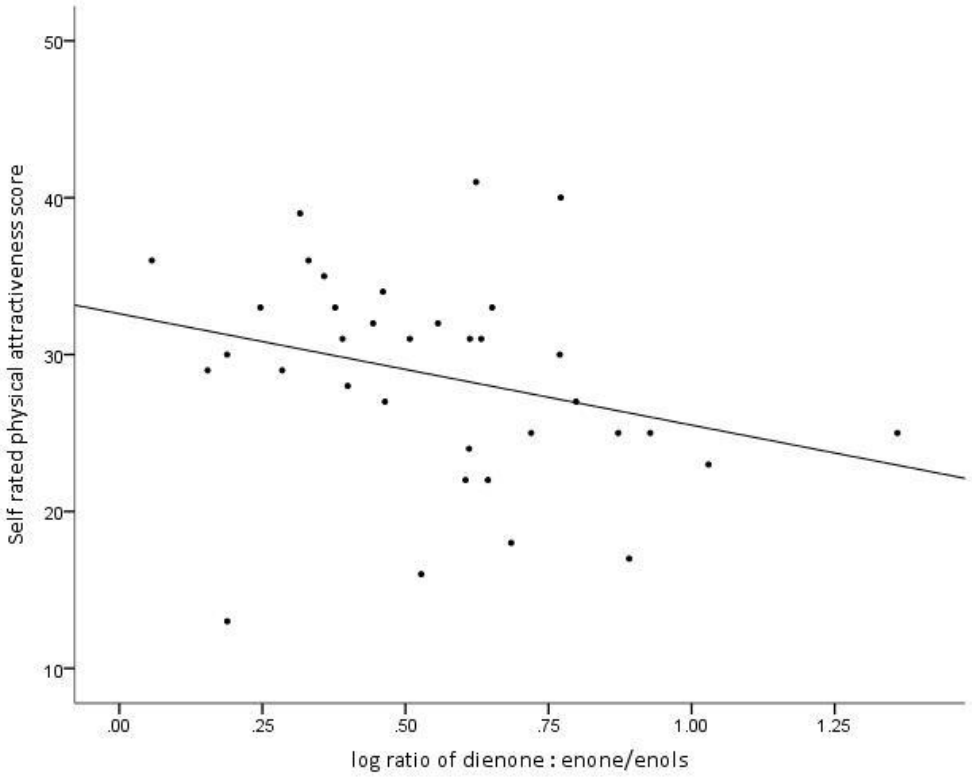


Figure 5.7 A logged ratio of –dienone /–enone-enol compounds negatively correlated with the men's self perceived physical attractiveness scores, as rated by a 9 item scale (r_s = -.376, p = .026).

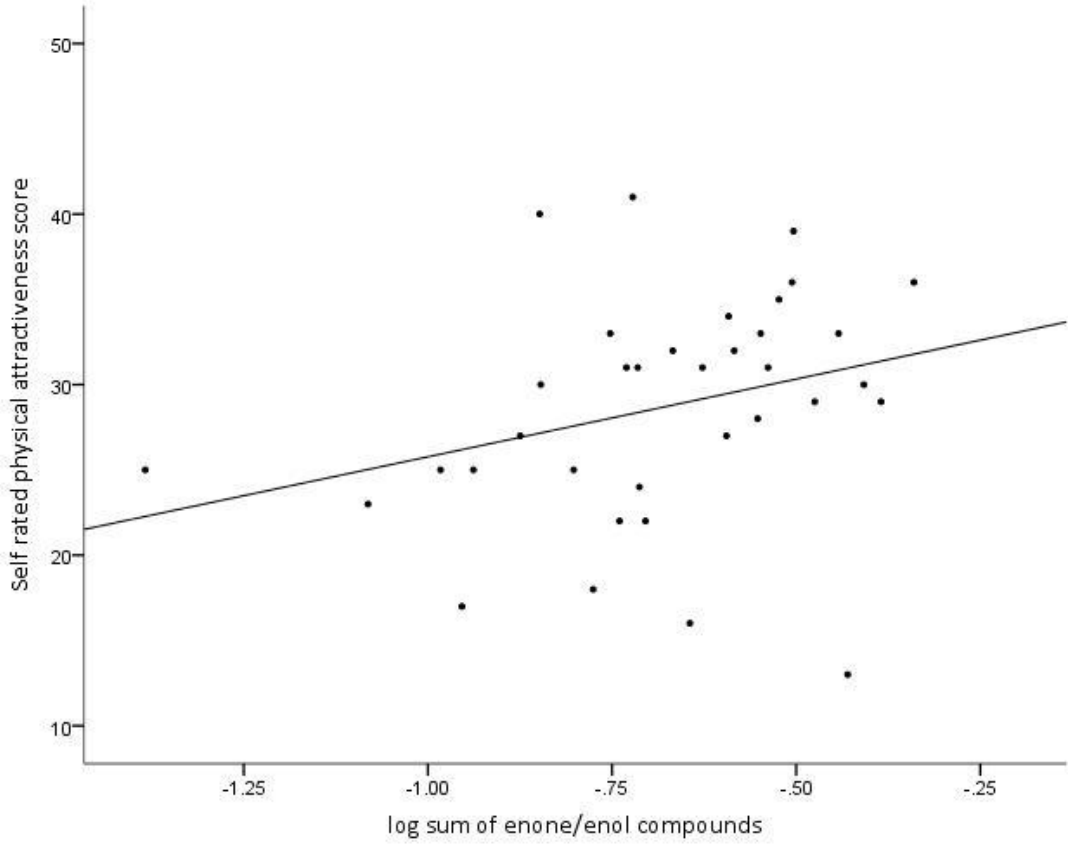


Figure 5.8 The logged sum of –enone and –enol compounds positively correlates with the men's self-perceived physical attractiveness scores, as rated by a 9 item scale (r_s = -.376, p = .026).

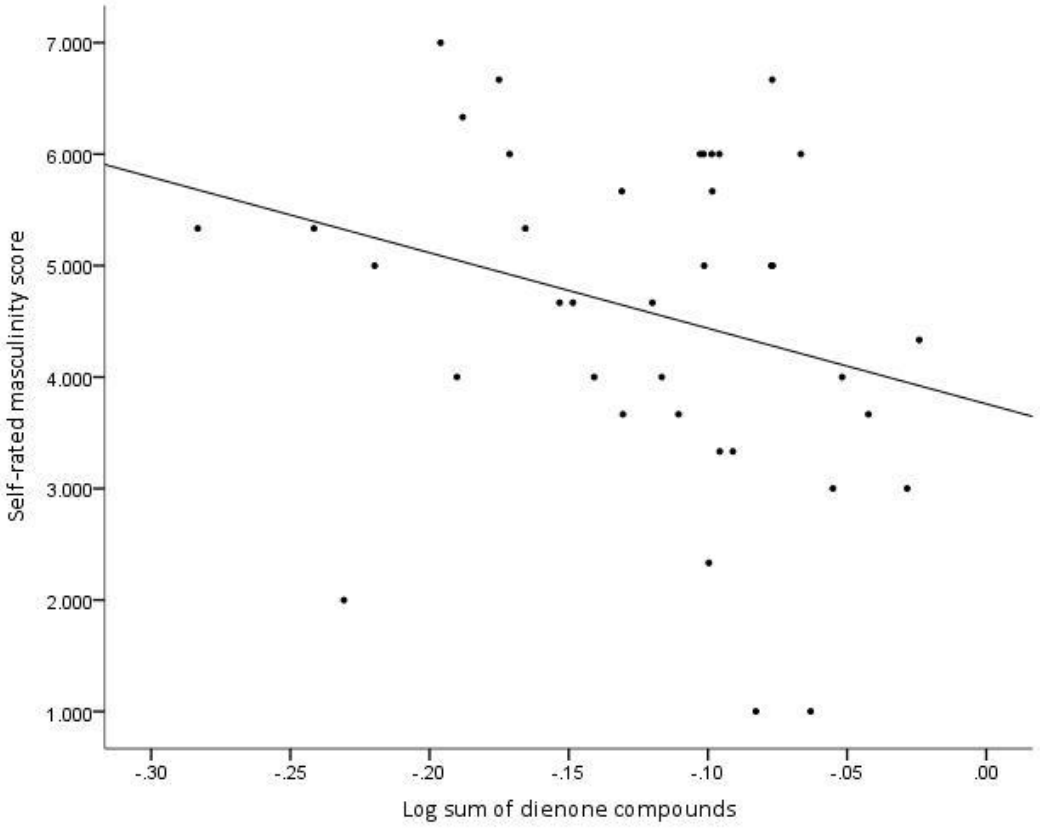


Figure 5.9 A logged measure of dienone compounds negatively correlates with men's self-rated masculinity scores (r_s = -.321, p = .049).

Table 5.3. Spearman's rank correlations of compound abundances with personality scores, attractiveness and masculinity ratings, and morphometric measurements.

	Sum of dienones		**Sum of enone and enols**		**Dienone : enone and enols**	
	r_s	***p***	**r_s**	***p***	**r_s**	***p***
Self rated personality scores						
Physical attractiveness	-0.296	0.071	0.374	**0.027***	-0.376	**0.026***
Assertiveness	-0.219	0.187	0.284	0.098	-0.286	0.095
Dominance	-0.069	0.680	0.077	0.659	-0.082	0.640
Valour	0.015	0.929	0.062	0.722	-0.064	0.715
Leadership	-0.024	0.886	-0.035	0.843	0.036	0.838
Big 5 personality scores						
Extraversion	0.059	0.726	-0.074	0.672	0.076	0.663
Agreeableness	0.088	0.597	-0.104	0.551	0.110	0.528
Conscientiousness	-0.150	0.370	0.046	0.793	-0.041	0.817
Emotional stability	-0.145	0.384	0.135	0.438	-0.141	0.418
Openness to experiences	-0.146	0.381	0.266	0.122	-0.271	0.115
3 item scales						
Attractiveness	-0.271	0.100	0.340	**0.046***	-0.340	**0.046***
Masculinity	-0.321	**0.049***	0.245	0.157	-0.248	0.150
Facial ratings by others						

Masculinity by females	-0.154	0.357	0.056	0.750	-0.057	0.743
Masculinity by males	-0.162	0.332	0.064	0.714	-0.065	0.711
Photo attractiveness by females	0.041	0.809	-0.062	0.725	0.060	0.734
Video attractiveness by females	-0.061	0.717	0.022	0.901	-0.017	0.922
Morphometrics						
Height	-0.113	0.499	0.080	0.647	-0.082	0.638
Weight	-0.190	0.253	0.158	0.365	-0.162	0.354
BMI	-0.178	0.284	0.168	0.335	-0.170	0.328
Chest	-0.209	0.207	0.149	0.392	-0.147	0.399
Hip	-0.101	0.547	0.089	0.611	-0.093	0.594
Waist	-0.033	0.844	0.038	0.829	-0.036	0.837
Shoulders	-0.346	0.033*	0.268	0.119	-0.269	0.118
Chest : hip ratio	-0.029	0.862	-0.032	0.854	0.037	0.833
Shoulder : hip ratio	-0.108	0.517	0.034	0.845	-0.030	0.864
Chest : waist ratio	-0.175	0.292	0.115	0.510	-0.116	0.507
Shoulder : waist ratio	-0.145	0.384	0.072	0.680	-0.074	0.672
Right 2D:4D	0.075	0.653	-0.042	0.811	0.041	0.813
Left 2D:4D	0.085	0.610	-0.095	0.588	0.094	0.591
Right and Left 2D:4D	0.075	0.657	-0.054	0.758	0.054	0.760

5.3.5 Identification of compounds

In the analyses to date, I have referred to the 8 androstene profiles as *-dienones*, *-enones* and *-enols*. Identification of the compounds has been attempted using mass spectrometry libraries for compounds with comparable molecular mass, but to date it has not been possible to definitively identify these compounds. There is one exception to this, namely androstenol (5α-Androst-16-en-3α-ol). However, this compound occurred in quantities which are relatively invariable (Fig. 5.1) and because of this, and

the fact that no signal was produced for this compound from a relatively large proportion of the sample, it was not included in the PCA or cluster analyses described above (null signal could indicate genuine absence of the compound or failure to detect small quantities). Identification of these compounds remains a task for the future, and ultimately, this necessitates validation through synthesis of the compound.

However, even pending this identification step, this study was able to make one further significant and surprising advance. In this comparatively large sample of men (the largest sample in a study of this kind), the compounds androstenone (5α-Androst-16-en-3-one) and androstadienone (androste-4,16-dien-3-one) were not present in any single individual. This was confirmed using pure standards run according to the same protocol in the same GC column: these analyses demonstrate the expected peak in the GC profile at the appropriate retention times. However, there were no peaks at these retention times (i.e. no compounds detectable at the same molecular mass) in any one of the 41 samples.

5.4 Discussion

The results indicate that male odour profiles for androgen steroids group into two clusters: 1. those having high concentrations of *-enols* and *-enones*, but low levels of *-dienones*; and 2. those having low levels of *-enols* and *-enones* but high concentrations of *-dienones*. The best social predictor of cluster membership is whether males are single or in a relationship, with cluster 1 being composed mostly of single males, and cluster 2 being comprised of those in relationships. Furthermore, independent rater scores of hedonic odour preferences indicate that those in cluster 1 (mostly single) have more pleasant and desirable odours than cluster 2 males (mostly paired).

5.4.1 Cluster membership

5.4.1.1 Links to relationship status

A more detailed examination of cluster membership showed that some of the paired males had odour profiles more similar to cluster 1 (single males) than we might expect. This overlap tendency can be partly explained by the men's relationship duration, in that outlier males were all in a relationship for a comparatively long period of time (typically over two years). Perhaps the most likely explanation is that this may be due to fluctuating levels of testosterone. Androgen steroids are thought to be derived from the metabolic break down of testosterone (1986a, 1987; Nixon *et al*., 1984, 1986b; Rennie *et al*., 1989); a male sex hormone. Testosterone levels can be affected by a variety of environmental factors, notably during new relationships, where T-levels are found to decrease (Gray, 2003; Marazziti and Canale, 2004). Similarly, paired men with lower baseline measures of testosterone are significantly more likely to still be partnered at a follow-up meeting (van Anders and Watson, 2006). It is perhaps unsurprising then, that one of the best social predictors of androstene content in males is explained in relationship status measures. Could the variation in testosterone levels amongst paired and single males be influencing the ratio of androgen steroid by-products in their odour profiles? Regrettably, no hormonal measures were taken from the males, so this theory would require further testing.

5.4.1.2 Links with hedonic T-shirt ratings psychometric scales

Cluster grouping may also be explained by self-ratings of physical attractiveness. Correlation results reveal that the higher the concentrations of *–enone*/*-enol* compounds, the more attractive the men rate themselves to be. The reverse is true with *-dienone* compounds, whereby attractiveness scores decrease with increasing concentrations. These results are consistent with hedonic ratings of the men's T-shirts, which show that males with higher *-enone*/*-enol* amounts were perceived as smelling more pleasant. Self-rated masculinity was also found to decrease with the increase of *-dienone* compounds,

yet this did not extend to the masculinity ratings given by others when they rated their photo. Indeed, none of the photo or video attractiveness and masculinity scores correlated with odour content.

Hedonic ratings of the T-shirt samples were found to differ between clusters, with those in cluster 1 smelling significantly more pleasant to both men and women. Furthermore, the cluster 1 T-shirts were also judged more desirable by female raters than the cluster 2 T-shirts. It is important to note that there was no significant difference in intensity ratings between the two clusters, implying that the results are not driven by a simple odour intensity effect, where stronger odours smell more unpleasant for example.

There was no correlation between hedonic ratings of the men's T-shirts and their photo and video attractiveness ratings. This is partly consistent with Roberts *et al.* (2011), who did not find a correlation with static ratings, however they did report a correlation between odour ratings and attractiveness of non verbal behaviour (displayed in videos). The reason for this discrepancy in results is unclear. There are various methodological differences between the studies which may have contributed to the different findings. For example, female raters in the current study did not smell the entire range of T-shirts, yet they did rate all of the male visual stimuli. Furthermore, information about hormonal contraception information was not collected and controlled for, as done by Roberts *et al.*. On the other hand, the relationship status of male donors in Roberts *et al.*'s study was not collected; a factor shown in the current study to be implicitly linked to hedonic odour ratings.

5.5 Final conclusions

This study aimed to link the underlying chemistry of human odour with recognized indicators of quality. The findings reveal an intriguing difference in axillary odour, and androstene profiles within the odour of paired and single men. Potentially, these differences in odour quality might be perceivable and play a role in social interaction. Furthermore, correlations between androstene profiles and rated

attractiveness and masculinity provide the first evidence for these putative pheromones being informative of measures of male quality in non-olfactory traits. Such inter-correlations between androstene expression and non-olfactory indicator traits are expected and support the interpretation that androstene profiles may be biologically meaningful, socially relevant cues of male quality.

However, I made the surprising discovery that neither androstadienone nor androstenone were present in any individual man in our sample. This was surprising because previous studies have reported their presence in both female and particularly male samples (see Table II). Although it might be argued that absence of signal is less conclusive than demonstration of its presence, confidence in our finding comes from both the large sample size and the fact that the same equipment and techniques did demonstrate a clear and obvious presence when the standards were injected into the GC column. The results therefore appear to be a true rather than false negative. How can we then explain the discrepancy between our finding and those of previous studies? There seem to be two possibilities. The first is that there is a difference in samples between studies, but this seems unlikely given that most previous studies were done in the same country and that the compounds were detected in most individuals in previous samples but not a single individual in this, (much larger) sample. The second possibility is that differences in sensitivity of equipment may be responsible. This appears more likely, given the interval between this and most previous studies (done in the 1980's and early 1990's) and the rapid pace of development of GC equipment. Thus, it seems likely that compounds identified as androstenone and androstadienone in previous studies were in fact not these compounds, but structurally similar ones that have not yet been identified and described. More work is needed to ascertain the identity of these compounds.

The finding that these two compounds are not in fact constituents of male body odour, at least not in our sample, is also important from the point of view of the study of their putative effects. A very large number of studies have been conducted over the past three decades, primarily using these two compounds, to investigate psychological and physiological influences in humans. The underlying, and

usually explicit, assumption on which these studies are based is that these compounds occur naturally in male body odour. The discovery that these compounds may not actually be present potentially undermines this entire body of work. However, in view of the fact that many of the studies report effects on the measured variables, including neural activation in the brain (Savic *et al*., 2001; Savic *et al*., 2005) and production of cortisol (Wyart *et al*., 2007), it seems more likely that these two compounds may be sufficiently structurally similar to the ones we in fact detect and quantify that they have equivalent effects – in other words, they may be functionally equivalent.

Notwithstanding this, however, the results of this study also indicate that these androstenes seem to occur in complex mixtures, with certain ratios between groups of compounds characterising different aspects of social status. The finding that biologically meaningful olfactory information is cued by ratios between different compounds, rather than absolute concentration of individual single compounds, is consistent with much of the animal literature on pheromonal communication. For example, colony-specific and individual recognition olfactory cues in ants are characterised by ratios between small numbers of cuticular hydrocarbons (D'Ettorre and Heinze, 2005), while the distinctive urinary odourtypes of MHC-congenic mouse strains are underpinned by variation in ratios of a limited number of volatile carboxylic acids (Singer *et al*., 1997). This hitherto unreported complexity may go some way in explaining the somewhat contradictory results with single compound human studies so far. It is to this that we now turn in Chapter Six.

Chapter Six: Effects of analytically-informed androstene mixtures on attractiveness judgements.

6.1 Introduction

This chapter describes an investigation into the effects of two androstene mixtures on attractiveness judgements. The previous chapter demonstrated that men can be allocated to one of two groups using androstene profiles in their axillary odour, and that group membership is partly explained by the relationship status of the men. Single men were found to have higher amounts of *-enone/-enol* compounds in their odour and were rated by females to have a more pleasant smell. In contrast, men in a relationship had fewer *-enones/-enols*, with *-dienone* compounds being more abundant; these odours were rated less pleasant by women. It was, therefore, hypothesised that the different types of male odour might cause cross-sensory modulation effects in females when viewing male faces. Furthermore, if results are consistent with previous chapters, it is also expected that suppressing effects might occur in men following odour exposure.

To my knowledge, this is the first study to use a mixture of androstenes as the odour stimulus rather than a single compound. Although previous studies using single compounds have yielded valuable results, there are contradictory findings that suggest different compounds may be having different effects. For example, androstenol has been reported to cause a rise in irritability in menstruating women (Cowley *et al*., 1980) and increased submissiveness mid-cycle (Benton, 1982)

whilst androstenone increased alertness and excitability (Kirk-Smith *et al*., 1990). On the other hand, androstadienone has been reported to make women feel more focussed (Lundström *et al*. 2003) and increase their positive mood as well as sexual arousal (Wyart *et al*., 2007). With a greater understanding of relevant compounds, it may be possible to explain some of these differing effects reported previously.

6.1.1 Rationale

To rightfully consider the 16-androstenes as pheromones they should, by definition, elicit a response in another individual. In this study, this prediction is addressed by using the specific ratios of 16-androstenes found in Chapter Five (See Chapter Five, section 5.3). To test this, attractiveness judgements were recorded pre- and post-exposure to the odour mixtures. Given that body odours containing higher amounts of *-enone/-enol* compounds are perceived by others as more pleasant, it is expected that this odour mixture might have a positive effect on women's attractiveness judgements of men. On the same basis, the less desirable odour, containing more *-dienone* compounds, would be predicted to cause a decrease in female attractiveness judgements. Furthermore, following the evidence presented so far that suggests odour may be having a suppressive effect in males, we might expect, in men, decreased self-ratings of attractiveness with exposure to *-enone/-enol* mixtures.

6.2 Methods

6.2.1 Participants

Seventy-five individuals, aged between 19 and 49 participated in this study (males: n= 20, mean age (±SD) = 22.53 ± 4.3; females: n = 55, mean age (±SD) = 23.09 ± 7.09). All participants were in good health and were not suffering from nasal congestion.

6.2.2 Ethical Approval

Participation in this study was voluntary and written informed consent was obtained beforehand. Participants were told that the aim of the study was to investigate odours and personal judgements in general, but were given no information regarding the different odour stimuli to be used. This study was approved by the University of Stirling's Psychology ethics committee.

6.2.3 Odour stimuli

Odour stimuli were based on the results of the cluster analysis reported in Chapter Five. Taking into account approximate ratios of compounds in each cluster, synthetic solutions were designed to resemble variable androstene profiles. For *-dienone, -enone* and *-enol* compounds, androstadienone, androstenone and androstenol were used. A further two solutions were produced to provide controls; i.e. *-dienone* alone and *-enone/-enol* alone. A summary of each solution and the concentrations is found in Table 6.1.

All compounds were purchased from Steraloids.com (androstenol (5α-Androst-16-en-3α-ol, ≥98% purity, Sigma Aldrich, CAS=1153-51-1, MW=274); androstenone (5α-Androst-16-en-3-one, ≥98% purity, Sigma Aldrich, CAS=18339-16-7, MW=272.43); and androstadienone (androste-4,16-dien-3-one, ≥98% purity, Steraloids.com, CAS=794-58-9, MW=270.41). Propylene glycol was used as a solvent (≥99.5% purity, Sigma-Aldrich, CAS=57-55-6). Solutions were prepared and left to dissolve on an overnight stirrer and then refrigerated until time to use; at that point the mixture was left undisturbed for 30-minutes to return to room temperature.

Table 6.1 Summary of solution content and number of males and females in each group

Solution	Ratio (*-dienone : -enone : -enol*)	Amount of androstadienone (µl)	Amount of androstenone (µl)	Amount of androstenol (µl)	N of each sex per solution group
1	1 : 0.4 : 0.1	1667	667	167	M = 4 F = 16
2	1 : 0.16 : 0.04	2083	333	83	M = 4 F = 18
3	1 : 0 : 0	2500	0	0	M = 9 F = 7
4	0 : 4 : 1	0	2000	500	M = 3 F = 14

Note. Amounts of androstadienone, androstenone and androstenol were taken from three prepared stock solutions, each with a concentration of 250µM in propylene glycol.

6.2.3 Photographic stimuli

To assess attractiveness judgements, participants viewed 25 opposite-sex faces and rated them for attractiveness using a seven point Likert-type scale with the anchors "1 = very unattractive, 4 = average, 7 = very attractive". Mean scores from this test were then calculated to give a measure of opposite-sex attractiveness judgements.

To assess self-rated attractiveness, participants rated themselves in comparison to other faces. Here they viewed a set of 25 same-sex faces and were asked "how attractive would you rate yourself in comparison to this person" using a seven point Likert-type scale with the anchors "1= much less attractive, 4= about the same, 7=much more attractive". Mean scores from this test were then calculated to give a general measure of self rated attractiveness.

6.2.4 Procedure

The participants completed the two face-rating tasks in each experimental condition (odour and control). Ratings took place during a single session lasting approximately one hour, with the control condition at the beginning of the session and odour exposure taking place at the end. The study was not counter-balanced for order effects because the analysis aimed to compare change in responses across odour conditions.

The session was held in a lecture theatre where all participants could see the screen on which the images were displayed. Participants first judged attractiveness of opposite-sex faces, followed by assessment of self-rated attractiveness using the face comparison test. Psychometric scales were used to assess dominance (Goldberg, 1999) and Rosenberg's Self-esteem Scale (Rosenberg, 1965). The *attitude* subscale of the revised sociosexual orientation inventory (SOI) (Penke and Asendorpf, 2008) was also included to assess any changes in views about casual uncommitted sexual relations (Simpson and Gangestad, 1991). The questionnaire used in this study can be found in Appendix XI.

For the odour condition, participants were then divided into four groups, each receiving one of the four odour stimuli. The odour was applied to the philtrum by application with a cotton bud. Participants were exposed to this odour for 30-minutes, exceeding the minimum incubation period of six minutes necessary to observe effects on mood in previous experiments (Jacob and McClintock, 2000). Participants were then asked to repeat the tasks. They recorded their answers on check sheets which were collected at the end of the session; participants were asked not to discuss their answers during the session.

6.2.5 Analysis

All analyses were carried out using IBM SPSS Statistics 20. Participant numbers were considerably low (especially male numbers), therefore participants were grouped in terms of the level of *-enone/-enol* and *-dienone* exposure, with two comparison groups: those given solutions 1 or 4 were in the high *-enone/-enols* group and those with solutions 2 or 3 in the high *-dienones* group. To test for differences in effects of the two androstene groups, a repeated-measures ANOVA was performed with condition (odour/control) as a within-subjects factor and solution type (high *–enone/-enols* vs. high *-dienones*) as the between-subject factor. The first analysis tested for differences in the personality questionnaire results, which included self-esteem, dominance and the *attitude* subscale of the revised SOI (Appendix XI).

A second analysis tested for differences in attractiveness judgements, as measured by opposite-sex ratings and a self rated score from a same-sex comparison. Condition was used as a within-subject factor as above. Solution type and participant sex were included as between-subject factors.

6.3 Results

6.3.1 The effect of odour on psychometric scales

For female participants, there was no significant main effect of experimental condition (F (3,51) = 1.764, p = .166), but there was a significant interaction between condition and solution type (F (3, 51) = 3.013, p = .038). This was largely driven by the results from the self-esteem scale, where scores of the females in the high *–enone/-enol* group increased with odour exposure (F (1, 53) = 6.999, p = .011) (Fig. 6.1). There were no differences between conditions or solutions for other measures of

dominance ($F(1, 53) = .183$, $p = .671$) and results from the SOI-R attitude scale ($F(1, 53) = .764$, $p = .386$).

There was also no significant main effect of condition for the questionnaire scores of the male participants ($F(3,15) = .030$, $p = .993$), nor interaction between condition and solution type ($F(3, 15) = .115$, $p = .95$).

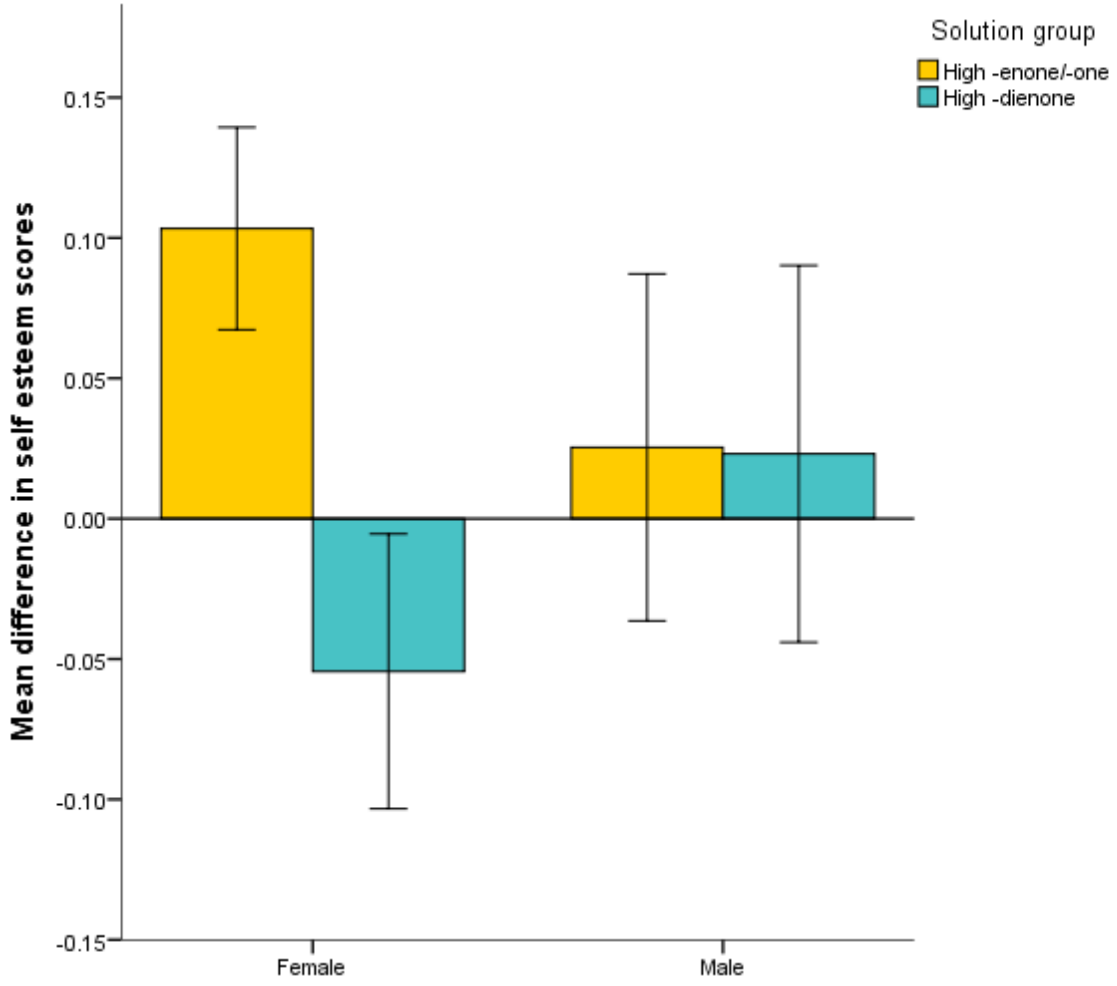


Figure 6.1. Mean difference in self-esteem scores (M±SEM). Bars represent the effect of different pheromone solutions. For females, there was a significant interaction for condition*solution ($p = .011$).

6.3.2 Attractiveness judgements

For attractiveness judgements of self and others, there was no significant main effect of condition (F (2,68) = .946, p = .393) or interaction of condition with solution type (F (2, 68) = .334, p = .717). However, there was a significant interaction effect between condition, solution type and participant sex (F (2, 68) = 4.360, p = .017). This was largely driven by changes in opposite-sex attractiveness ratings, where men in the high *–enone/-enol* group gave women significantly lower ratings compared to the control condition and to those in the high *–dienone* group; whilst women gave men higher ratings if they were in the high *–enone/-enol* group (F (1, 69) = 4.208, p = .044) (see Fig. 6.2).

For same-sex attractiveness ratings, there was no significant interaction effect of condition with solution type and participant sex (F (1, 69) = 2.430, p = .124). Yet visual inspection of the data suggest that, for those in the high *–enone/-enol* group, there was a tendency for women's self ratings to increase and men's self ratings to decrease (Fig. 6.3). For women in the high *–enone/-enol* group, the tendency for self ratings to increase compared to the control is significant (paired samples t-test, t (28) = -2.408, p = .023).

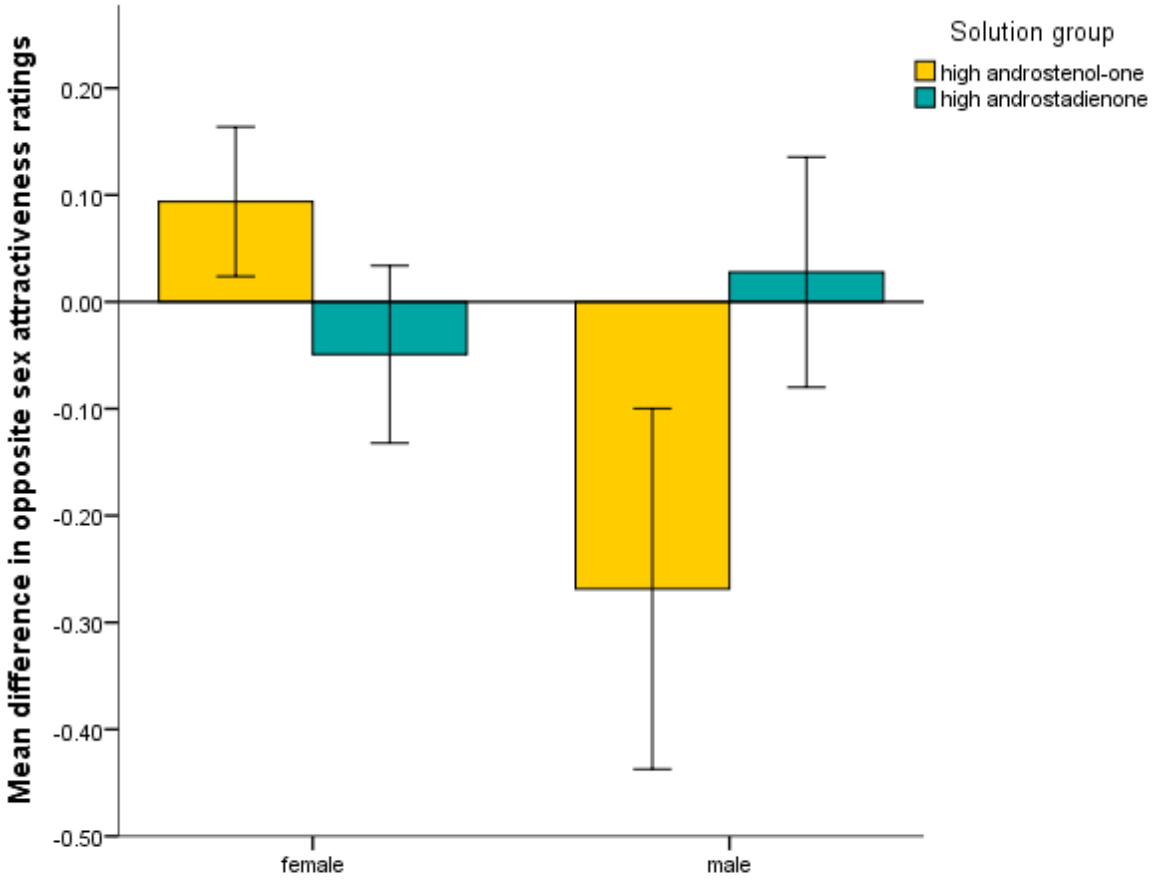


Figure 6.2. Mean difference in opposite sex attractiveness ratings, across pheromone solution group. (M±SEM) There was a significant interaction between condition* solution * participant sex (p = .044).

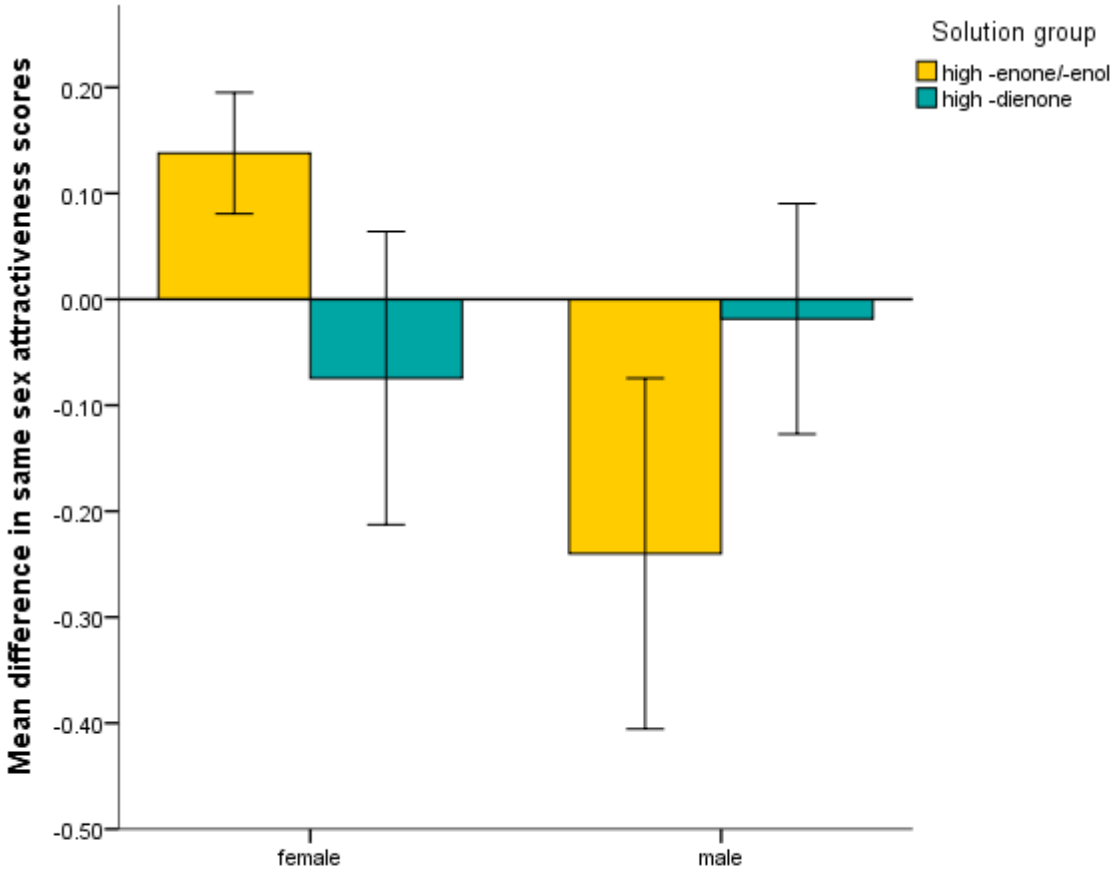


Figure 6.3. Mean difference in self rated attractiveness, as measured by a same-sex comparison. There was a near significant interaction between condition* solution * participant sex (p = .124)

6.4 Discussion

Based on the results reported in Chapter Five, I aimed in this experiment to test the effect of specific androstene mixtures on self-perception and attractiveness judgements. The findings suggest that high -*enone/-enol* mixtures may have gender-specific effects. In females, exposure is associated with increases in self-esteem and judgements of male attractiveness, while in males, exposure is associated with lower assessments of self-rated attractiveness and lower ratings of the opposite sex.

6.4.1 Psychometric scales

For females, high *–enone/-enol* solutions were found to be associated with an increase in self-esteem, whereas those exposed to high *–dienone* solutions tended to have reduced scores. This effect could be described as modulatory (Jacob and McClintock, 2000), with exposure being linked to increased positive emotions that may have secondary effects in mate choice decisions. Furthermore, this is also what we might expect given the prediction that more preferable male odours might have positive cross-sensory modulation effects when viewing male faces.

For males, there was no significant difference between conditions or pheromone solution type for scores on the self-esteem and dominance scales, nor the *attitude* subscale of the revised Sociosexual Orientation Inventory. This suggests that the odour-induced changes in attractiveness ratings (described below) are not explained by changes in general mood or social attitudes and appear to be specific to facial judgements. These findings are comparable with those described in Chapter Two, where there was no change in self-esteem and only a shift in general mood and dominance when analysed together. The direction of this effect was that general mood decreased and feelings of dominance increased in both men and women. Because a general measure of mood was not taken for this study, a direct comparison is not possible. Yet it is clear that the main effects to be found in each dataset are specific to facial judgements and behaviour in a mate choice context.

6.4.2 Attractiveness judgements

6.4.2.1 Opposite-sex ratings

There was a significant interaction between condition, solution type and participant sex for attractiveness judgements, largely driven by opposite-sex scores. Men exposed to the high *–enone/-enol* solutions rated women as less attractive compared to the control condition and the high – *dienone* group, whose scores were no different from the control. This result was unexpected, and it is not immediately clear why the more preferable odour of single men might be associated with men giving lower attractiveness scores to women. Perhaps this could be explained with the general suppressive male odour hypothesis recurring throughout this thesis. That is, the effect of male odour on other men may extend beyond a reduction in self-perception, to contribute to a more generalized disinterest in mate choice interactions, such as the decrease in female ratings we see here. Certainly, similar interactions have been observed in animals where dominant male odours are found to suppress sexual activity in surrounding subordinates (Perret, 1992).

For women, those in the high *–enone/-enol* group showed a tendency to rate men as more attractive compared to the control condition. Again, high *–dienone* odours seemed to have no effect on attractiveness judgements; however, there was a tendency for ratings of men to decrease with high – *dienone* exposure. These findings marry with those from Chapter Five which indicate that odours containing more *-enone/-enol* compounds are preferred by females. It is also complementary to the hypothesis that androstene compounds might be acting as intersexual signals in humans and is comparable with previous literature which has reported positive effects of androstenol and androstenone on females (see Table I, Section One). They also support claims made in Chapter Two, which revealed a tendency for androstadienone to be associated with lower ratings of opposite-sex attractiveness.

On the other hand, these findings differ with Saxton *et al*.'s (2008) study where they found an *increase* in females' perceptions of attractiveness when they were exposed to androstadienone. However, the difference in results might be partly explained by considerable differences in methodologies employed in both studies. While Saxton *et al*. (2008) aimed to achieve high ecological validity with their face-to-face experimental design, it is possible that meeting the males in this way also altered the context of the study which, in turn, may have interfered with the way that the odour was perceived.

Cornwell *et al*. (2004) found concordance between olfactory and visual stimuli ratings, but only in the context of a long-term relationship. That is to say, women's preferences for masculinised faces correlated with higher ratings of androstadienone when asked to base their preferences in the context of a long-term relationship. It may be the case that if the women in Saxton *et al*.'s study were rating the men as potential dates, they may have been making their decisions based on the context of a long-term relationship. This could be related to the results from Chapter Five, which demonstrate that men in a relationship tend to have higher amounts of *-dienone* compounds in their odour; potentially giving rise to the increased preference for androstadienone seen in Cornwell *et al*. (2004) and the increase in opposite-sex ratings seen in Saxton *et al*. (2008b). Although the female odour preference in Chapter Five was directed towards *-enol/-enone* compounds, this measurement was a simple pleasantness rating and not given the context of short or long-term relationships.

There may also be some debate over using computer-displayed facial stimuli in odour studies, for fear of lacking ecological validity. Yet there have been many reports of this experimental method being sufficient to induce effects (for a review, see Havlicek *et al*., 2010). Meeting the males face-to-face may also alter the context of study, which in turn may interfere with the way the odour is perceived.

6.4.2.2 Same-sex ratings

When men rated their own attractiveness in comparison to other male faces, there was a non-significant tendency for those who were exposed to the high *-enone/-enol* solutions to feel less attractive than they did in the control condition. Men who were exposed to the high *-dienone* solutions felt no change in self-perceived attractiveness compared to the control condition. These findings are largely consistent with previous results in this thesis which suggest that, in general, the presence of male odour and its components may have a suppressive influence in males.

The results are also in keeping with previous studies that examined the effect of *-enone/-enol* compounds on feelings of attractiveness. Decreases in self-perceived attractiveness were reported after exposure to androstenone and androstenol independently (Filsinger *et al*., 1985; Filsinger *et al*., 1984). For male subjects, androstenol and androstenone appeared to raise attractiveness judgements of a target male stimulus whilst their ratings of their own sexiness decreased.

For females, self-rated attractiveness scores increased with exposure to high *-enone/-enol* solutions. This is consistent with the change in self-esteem associated with these solutions, that is to say, women exposed to high *-enone/-enol* odours were found to have increased self-esteem and feel more attractive. Similarities can be drawn here with early reports of positive effects of androstenol and androstenone on women, which are summarized in Table I, Section One.

The mechanism behind this shift in self-perceived attractiveness may be explained by studies that have looked at the neuronal consequences of exposure to androgen steroids. Savic *et al*. (2001) found that inhalation of androstadienone activates the fusiform gyrus, a brain region that is involved in the visual processing of faces (Tarr and Gauthier, 2000). Other work has demonstrated general hypothalamic activations through exposure to androstenol (Savic and Berglund, 2010) and androstadienone (Berglund *et al*., 2006; Savic *et al*., 2005). These studies can be linked to the present one by Kovacs *et al*. (2004), who demonstrated that another androgen steroid (5-α-androgenst-16-en-3-

one) caused males to perceive faces as more masculine. In the context of the present study, it therefore seems plausible that men exposed to the odours may have perceived the stimuli faces as more masculine and felt comparatively less attractive as a result.

Although the present findings seem to be complementary with the previous results from Chapter Two and also the suppressing effect of male odour on male performance in Chapters Three, Four and Five, the small sample sizes of the groups mean that these results should be treated with some caution. More research on this topic would be beneficial to truly understand sex-specific modifications in facial judgements following exposure to this analytically-informed mixture of androstenes.

6.5 Final conclusions

The results from this study point towards a quality-dependent odour signalling system in humans, with gender and context-specific responses taking place. Using analytically informed mixtures of compounds based on the results from Chapter Five, it was possible to test the effect of the more preferable odour profile characteristic of single men against that characteristic of those in a relationship. As predicted, there was evidence to suggest that the "single-male odour" enhanced women's attractiveness judgements of male faces whilst potentially decreasing feelings of self-perceived attractiveness in men (although this was non-significant). On the other hand, the odour typical of partnered men was found have no significant effect on women's attractiveness judgements of male face, although there was a tendency for ratings to decrease with odour exposure. The endocrinological basis of this effect may be centred around circulating testosterone levels in males, as discussed below.

The findings also support the results from previous chapters, which indicate a suppressive effect of male odour in other men. Whether the result could be interpreted in terms of a quality effect, with males experiencing feelings of subordination as a result of exposure to a higher quality odour,

remains to be seen. In Chapter Five, correlations of odour content with various indices of quality yielded few associations, yet despite this an assumption of quality on the basis of differing testosterone levels could be argued. There have been various reports of drops in testosterone for males at the beginning of a relationship (Burnham *et al*., 2003; Gray, 2003; Marazziti and Canale, 2004); with these results it could be assumed that single men have higher circulating levels of testosterone; a compound associated with male quality (Archer, 1991; Christiansen, 1998). A useful measure in this case for future work might be partner history, using reproductive success to distinguish between single males who are attractive and between relationships and those who have been single for a long-term, perhaps because they are less attractive to women.

The use of androstenone, androstenol and androstadienone in this study enabled a comparison of results with existing literature on these compounds, whilst offering a new perspective through using them in mixtures based on real male odour. Although the GC-MS analysis from Chapter Five revealed that '*dienone*-type' compounds were present in the odour samples, there was no indication that androstadienone was present. This may lead to the possibility that androstadienone might not be a biologically relevant compound in this study. However, the same reservations could be levelled for the use of androstenone and androstenol as the *-enone/-enol* compounds, which were also not fully isolated in our analysis. Therefore, the results in this chapter do not provide definitive evidence of human chemosignals in action, although they are consistent with what might be expected. Further investigations should trial similar *-dienone*, *-enone* and *-enol* compounds, pending identification and isolation of the actual compounds present in odour.

In summary, this is the first study to test the effect of androstene mixtures on attractiveness judgements based on an up-to-date assessment of male body odour. It may also shed light on previous equivocal results that show different effects for each androstene compound, or no effects at all. For example, the varying effects of androstadienone in women might be explained by the context of the study in question; whether it is in the context of a short-term or long-term relationship. It is structurally

similar to compounds found in the less preferable odour of partnered men, yet, in the appropriate context, this may have more positive effects on judgements of attractiveness. Finally, this study highlights the importance of androstene choice in human odour-signalling studies, revealing intricate compound and sex specificity which will hopefully be considered in the design of future experiments in this field.

Analytical assessment of human axillary odour – an evolutionary approach: general discussion.

In this section, I focussed on the chemical underpinnings of human odour and asked a fundamental question in human chemo-signal research: do variations in individual odour content correlate with indices of phenotypic quality? The study in Chapter Five aimed to answer this question with an analytical assessment of male odour via GC-MS analysis. The results indicated that odour profiles for androgen steroids can be grouped into two clusters: 1. those having high concentrations of *-enols* and *-enones*; and 2. those having low levels of *-enols* and *-enones* but high concentrations of *-dienones*. The best social predictor of cluster membership was whether males were single (cluster one) or in a relationship (cluster two). Furthermore, hedonic ratings of T-shirts from cluster one males were found to be more pleasant than those from cluster two. This study was the first to combine analytical techniques with human evolutionary theory by correlating androstene abundance with aspects of phenotypic quality and socially relevant traits.

The findings from Chapter Five confirm the existence of androgen-type steroids in human odour and make substantial contributions to explaining inter-individual variation in their occurrence, suggesting that variability may be due to social determinants implicitly linked with fluctuating testosterone levels. If androstene production is mediated by testosterone metabolism (1986a, 1987; Nixon *et al*., 1984, 1986b; Rennie *et al*., 1989), and circulating testosterone levels are influenced by relationship status and duration (Gray, 2003; Marazziti and Canale, 2004), the correlation found in this

study seems feasible. However, testosterone levels are highly variable and can be influenced by a range of environmental factors beyond relationship statuses, such as aggression (e.g. 1987; Dabbs and Morris, 1990; Ehrenkrantz *et al*., 1974; Kreuz and Rose, 1972). Therefore, without an extensive hormonal investigation, the association between male odour profiles, relationship status, and testosterone levels cannot be certain.

A further understanding into metabolic pathways would also be beneficial in interpreting relative androstene ratios. There is evidence to suggest that androstenol and androstenone are the breakdown products of androstadienone biotransformation by corynebacteria (Austin and Ellis, 2003). Under the assumption that the compounds indentified in Chapter Five are chemically similar to the 16-androstenes, could the ratio of *-enols* and *-enones* to *-dienones* be explained by the activities of axillary corynebacteria? Further investigations into the complex biochemical pathways of the skin microflora will be essential in answering this question.

Pheromone definitions state that the compound(s) in question must elicit a response in a member of the same species (Bronson, 1971; Karlson and Luscher, 1959). The following question was therefore raised: will exposure to high *-enone*/*-enol* odours elicit cross sensory modulation effects in the receiver? This question was the focus of Chapter Six, which found sex-specific responses to the "single-male odour", where exposure was associated with an increase in women's self-esteem and opposite-sex attractiveness judgements. While men exposed to this odour rated women less attractive, an effect that might be interpreted as disinterest. Furthermore, men showed a non-significant tendency to rate their own attractiveness as lower in the high *-enol*/*-enone* odour exposure, consistent with previous findings in this thesis which suggest body odour to be having a suppressive effect in males.

Together, the results from this section go some way in explaining previous equivocal findings in androstene research, where different compounds and contexts are used across experiments. For example, if high *-dienone* odours are associated with males in a relationship, then perhaps findings will

depend on the context of the study and the preferences of the female at that time. Indeed, Cornwell *et al*. (2004) found that women's preferences for masculinised faces correlated with higher ratings of androstadienone, but only when asked to base their preferences in the context of a long-term relationship. This reinforces the prediction that females are sensitive to the context of mating (Little *et al*., 2002), perhaps in multiple modalities.

In summary, despite the exact identity of the compounds responsible for cluster membership being so far unknown, the findings in this section offer a new insight into how the androstenes might be relevant in humans and highlight the need for more research, across disciplines, to gain a comprehensive understanding of the functional significance of the androstenes, from the underlying variation between individuals in androstene precursors (ultimately, testosterone), the chain of biochemical events that shape androstene profiles through bacterial metabolism of testosterone derivatives at the skin surface, regulation of these processes by social context, and finally the adaptive benefits that arise through body odour perception.

Chapter Seven: An optimistic future for human odour research.

The data chapters presented in this thesis have described experiments that examined the role of odour in human social interactions. The evidence was categorised on the basis of two selective pressures; inter- and intra- sexual selection. Although these mechanisms are not mutually exclusive, they help to provide a framework for results to be displayed and so form the theme of the first two sections of this thesis. The third section hinges on understanding the chemical basis of human odour, although the two signalling theories remain integral in the interpretation of results. The experimental findings were discussed in detail at the end of each chapter and in the discussion of each section. In this chapter, I return to the fundamental themes of the thesis, equipped with new perspectives gained from the results.

7.1 Human odour – inter-sexual or intra-sexual signals?

The results of this thesis provide recurrent evidence to suggest males play a more important part in human odour interactions than researchers might have previously interpreted, raising the question of whether they might be 'receivers' as well as signallers. I have replicated the findings of androstene-induced decreases in self-perceived attractiveness in males (Filsinger *et al*., 1985) and extended the investigation to include a dynamic video assessment, finding that AND-exposed males also behave less attractively, as judged by third-party raters (Chapter Two). Partially repeating this assessment with exposure to a 'single man' odour mixture, a similar trend of decreased self-perceived

attractiveness was revealed, as well as an apparent disinterest in females (as interpreted by lower opposite-sex attractiveness ratings) (Chapter Six). Furthermore, I have shown that variations of contextual male odour taken from a competitive context may suppress aspects of physical performance in men (Chapters Three and Four).

There is also strong support for the idea that odour may be acting as an inter-sexual signal for females. This statement stems from the evidence that male odour profiles can be explained by relationship status, with single men's odours generally being found to be more attractive than the odours of paired men (Chapter Five). The chemical basis of this phenomenon is discussed later, but at this point it is necessary to refer to the finding reported in Chapter Six, where exposure to the androstene mixtures based on the 'single man odour' led to an increase in women's self-esteem and self-rated attractiveness scores, as well as a tendency to rate male faces as more attractive. I also found evidence to suggest that females exhibit behavioural responses to contextual male odour, where stimulatory reactions to competitive odours contrasted with reactions to neutral odours (Chapter Four).

Examples of intra-sexual and inter-sexual odour signalling have been presented, which leads us back to a question posed at the beginning of this thesis. There I asked which of these roles odour was likely to be implementing in humans, simply to raise the point that such a question would be beneficial during the experimental design and interpretation of results. Indeed, the studies in this thesis were not explicitly designed to 'rule-out' the possibility of one mechanism. Yet by including, for example, both sexes in the majority of these studies and by investigating new contexts, I have produced evidence that suggests both inter- and intra- sexual signalling may be relevant in humans. This idea is valid, as it is shown that a number of sexual selection mechanisms can act together in odour signals of the same species (Gosling and Roberts, 2001; Moore and Moore, 1999). Women, therefore, might respond to male odour cues of dominance and quality (as suggested in this thesis) as well as men, although this idea would benefit from further testing as it is likely that the association between male dominance and female preference is not so straightforward. For example, there may be

costs to mating with a dominant male if interactions bring with it exposure to increased aggression levels (Moore and Moore, 1999; Ophir and Galef, 2003).

7.2 The 16-androstenes – human pheromones?

In previous chapters, I have highlighted the range of qualities that make the 16-androstenes a unique and intriguing set of compounds. Sexual dimorphisms in expression, thresholds, and hedonic perceptions, combined with a plethora of reports identifying exposure effects, seem to be mostly consistent with the properties of sexually selected signals (Darwin, 1871). Despite this claim, general views on the 16-androstenes being pheromones remain largely negative, mainly due to inconsistent findings and the uncertainty of their definitive occurrence in human odour. It must be noted however, that the majority of these shortfalls are due to experimental issues over time, rather than a lack of effect (although this has been described in a few cases (Black and Biron, 1982; McCollough *et al*., 1981)). It is therefore hoped that by returning to equivocal results (Chapter Two) and making reassessments of abundances (Chapter Five), this thesis has stressed the need to persevere with androstene research.

Up until now, records of androstene abundances were vague and based on studies with small sample sizes. Furthermore, there has been no effort, as far as I am aware, to search for meaning within odour profiles, by correlating compound abundance with known indices of quality. Section Three addressed this issue, and found that odour profiles in males could be cueing information on relationship status. Specifically, single males were found to have more *–enone/-enol* compounds in their odour, whereas men in a relationship tended to have more *–dienone* compounds depending on their relationship duration.

With more known about individual variation in androstene abundances, future work might be able integrate these findings into their experimental designs. For example in studies adopting a mate choice context, it will be beneficial to know that *–enone/-enol* compounds may be most relevant in

short-term attractiveness contexts. Despite the fact that final identification of these compounds is still pending, the study in Chapter Five has made substantial headway on the road to understanding the complex chemical underpinnings of human odour profiles.

7.3 Principal findings

This thesis contains two principal findings that might have future implications in the field. First, is the recognition that intrasexual odour signalling may be occurring in men. This is supported consistently throughout the studies in this thesis, with the finding that male odour and its alleged components have a suppressive effect in men. By adopting an interdisciplinary approach, the possibility of male intrasexual signalling has been examined explicitly in a competitive context, which has to my knowledge, never been attempted. Therefore it is hoped that this body of work has perhaps formed a starting point for a new context in human odour research, paving the way for further investigation.

Second is the discovery that male odour profiles fall into two categories, which can be explained by relationship status. In essence, single men will likely have different androstene-type compounds in their odour compared with men in relationships. Furthermore, the odour of single men is generally found to smell more pleasant to females and a synthesised mixture based on this odour is found to have positive psychological effects in women. Could it be that the odours of (high quality) single men effectively 'advertise' their availability to women, eliciting positive changes in mood that may extend to increased attractiveness judgements? This would of course be an adaptive strategy for single men, and evidence showing that androstenes are derived from testosterone metabolism suggests that this may turn out to be an honest signal (Archer, 1991; Christiansen, 1998; Grossman, 1985; Zahavi, 1975). However, until we have specific knowledge of the compounds involved, this finding, for now, remains a well-informed prediction.

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Appendix I

Medical History Questionnaire

Trial Supervisor _____

HOL/HOR reference _____

Panellist Number _____

New Panellist Yes/No

Subject initials _____ DOB _____

Before beginning any study we need you to complete and return this form. Please circle the appropriate response. A yes answer does not mean you will be excluded from a study.

If you answer yes to any of the questions below please give us full details to every yes answer either on the back of this form or on the medication chart (question 2).To enable us to make an accurate decision it is important to have as much information as possible.

1. Are you attending or receiving regular treatment from a Doctor?	Yes	No
2. Are you taking or using any medication, pills, tablets, ointments, injections or inhalers, either from your doctor or on your own accord?	Yes	No
3. Are you allergic to or have you ever had any unfavourable reaction to		
a) Medicines?	Yes	No
b) Food?	Yes	No
c) Cosmetic or personal product?	Yes	No
d) Any other substance?	Yes	No
4. Have you been unwell over the last 7 days?	Yes	No
5. Do you suffer from hay fever?	Yes	No
6. Have you ever suffered from asthma or any respiratory disease?	Yes	No
7. Do you have nasal polyps or allergic rhinitis?	Yes	No
8. Do you suffer form migraines or severe headaches?	Yes	No
9. Do you suffer from epilepsy or other forms of fit?	Yes	No
10. Do you suffer from any form of visual disturbance?	Yes	No

11. Do you suffer from carpal tunnel syndrome or repetitive strain injury? Yes No

12. Do you currently have any trauma to or disability in your arms, hands or fingers? Yes No

13. Do you suffer from shaking or tremors of any form? Yes No

14. Do you currently, or have you historically, suffer from affective or similar disorders e.g. depression, anxiety, stress? Yes No

15. Do you currently, or have you historically suffer from eating disorders or body image disturbances? Yes No

16. Do you currently, or have you historically suffer from psychiatric conditions Yes No

17. Is there anything else concerning your health you think we should know about? Yes No

If you have answered yes to any of the above please provide full details below.

The type of information we need to know is

- The name of the condition
- How long you have had it and timing of last attack/flare up
- Any treatment you are receiving for it (See medication chart)
- How you are affected by it.

_____

_____

_____

_____

_____

To the best of my knowledge this information is correct. I understand that this information may be inspected by authorised personnel and will be treated in strict confidence.

Initials…… Date ……

FOR OFFICE USE ONLY

APPROVED	NOT APPROVED	WHY

Signed ……. Date ……

Print Name……..

Possible exclusions for perfume studies.

1. Sensitivity to any of the ingredients.
2. Asthma
3. Nasal polyps
4. Allergic rhinitis
5. Anti histamines, Steroids and immunosuppressant meds.
6. ….
7. ….

Additional exclusions will be added according to SEAC advice over ingredients and possible side effect profiles

nued)
Concomitant Medication

Panellist number _____

Please detail any medication you are currently taking

Name of medication	Dose	Frequency (how often you take it)	Route *	Any side effects	Date Started	Date Stopped

* How you take the medication – e.g. by mouth, on the skin, injection, inhalers.

If you have any changes to your medication, or start any new medication, please give details on the form above.

OFFICE USE ONLY

Reviewed by	Date	Comments

Appendix II

Current Thoughts Scale

This is a questionnaire designed to measure what you are thinking at this moment. There is, of course, no right answer for any statement. The best answer is what you feel is true of yourself at this moment. Be sure to answer all of the items, even if you are not certain of the best answer. Again, answer these questions as they are true for you RIGHT NOW.

Using the following scale, place a number in the box to the right of the statement that indicates what is true for you at this moment:

1 = not at all
2 = a little bit
3 = somewhat
4 = very much
5 = extremely

1.	I feel confident about my abilities.	☐	P
2.*	I am worried about whether I am regarded as a success or failure.	☐	S
3.	I feel satisfied with the way my body looks right now.	☐	A
4.*	I feel frustrated or rattled about my performance.	☐	P
5.*	I feel that I am having trouble understanding things that I read.	☐	P
6.	I feel that others respect and admire me.	☐	A
7.*	I am dissatisfied with my weight.	☐	A
8.*	I feel self-conscious.	☐	S
9.	I feel as smart as others.	☐	P
10.*	I feel displeased with myself.	☐	S
11.	I feel good about myself.	☐	A
12.	I am pleased with my appearance right now.	☐	A
13.*	I am worried about what other people think of me.	☐	S
14.	I feel confident that I understand things.	☐	P
15.*	I feel inferior to others at this moment.	☐	S
16.*	I feel unattractive.	☐	A
17.*	I feel concerned about the impression I am making.	☐	S
18.*	I feel that I have less scholastic ability right now than others.	☐	P
19.*	I feel like I'm not doing well.	☐	P
20.*	I am worried about looking foolish.	☐	S

Note: The statements with an asterisk are reversed-keyed items
The letter in the last column indicates the primary factor on which that item loaded in a factor analysis. The three factors were labelled performance self-esteem (P), social self-esteem (S) and appearance self-esteem (A).

Appendix III

Physical Attractiveness Scale

1. Physical Attractiveness (Personal Attributes Survey)

Instructions: Below is a list of statements dealing with your general feelings about yourself. If you strongly agree, circle SA. If you agree with the statement, circle A. If you disagree, circle D. If you strongly disagree, circle SD

Scoring Instructions *(NB: not seen by panellists)*

1. *	I don't consider myself attractive	SA	A	D	SD
2.	I like to look at my body	SA	A	D	SD
3.	I attract attention from the opposite sex	SA	A	D	SD
4.	I like to look at myself in the mirror	SA	A	D	SD
5.	I am considered attractive by others	SA	A	D	SD
6.*	I dislike looking at my body	SA	A	D	SD
7.	I have a pleasing physique	SA	A	D	SD
8.	I like to show off my body	SA	A	D	SD
9.*	I dislike looking at myself in the mirror	SA	A	D	SD

SA=3, A=2, D=1, SD=0.

Items with an asterisk are reverse scored, that is, SA=0, A=1, D=2, SD=3.

Sum the scores for the 9 items.

The higher the score, the higher the self perceived physical attractriveness.

Appendix IV

Dominance Scale

1. **Dominance** (The Items in the 33 Preliminary IPIP Scales Measuring Constructs Similar to Those in Gough's California Psychological Inventory (CPI))

Instructions: Below is a list of statements dealing with your general feelings about yourself. If you strongly agree, circle SA. If you agree with the statement, circle A. If you disagree, circle D. If you strongly disagree, circle SD

1.	I try to surpass others' accomplishments	SA	A	D	SD
2.	I try to outdo others	SA	A	D	SD
3.	I am quick to correct others	SA	A	D	SD
4.	I impose my will on others	SA	A	D	SD
5.	I challenge others' points of view	SA	A	D	SD
6.*	I hate to seem pushy	SA	A	D	SD
7.	I am not afraid of providing criticism	SA	A	D	SD
8.	I put people under pressure	SA	A	D	SD
9.	I lay down the law to others	SA	A	D	SD
10.	I want to control the conversation	SA	A	D	SD
11.	I demand explanations from others	SA	A	D	SD

Scoring Instructions *(NB: not seen by panellists)*

SA=3, A=2, D=1, SD=0.

Items with an asterisk are reverse scored, that is, SA=0, A=1, D=2, SD=3.

Sum the scores for the 11 items.

The higher the score, the higher the self perceived dominance.

Appendix V

Competence (The Items in the 33 Preliminary IPIP Scales Measuring Constructs Similar to Those in Gough's California Psychological Inventory (CPI))

Instructions: Below is a list of statements dealing with your general feelings about yourself. If you strongly agree, circle SA. If you agree with the statement, circle A. If you disagree, circle D. If you strongly disagree, circle SD

1.	I am full of ideas	SA	A	D	SD
2.*	I am easily hurt	SA	A	D	SD
3. *	I excel in nothing at all.	SA	A	D	SD
4. *	I get confused easily.	SA	A	D	SD
5.	I come up with good solutions	SA	A	D	SD
6.*	I question my ability to do my work properly	SA	A	D	SD
7. *	I am easily offended	SA	A	D	SD
8.	I know how to apply my knowledge	SA	A	D	SD
9.*	I feel crushed by setbacks	SA	A	D	SD
10.*	I know that I am not a special person	SA	A	D	SD

Scoring Instructions *(NB: not seen by panellists)*

SA=3, A=2, D=1, SD=0.

Items with an asterisk are reverse scored, that is, SA=0, A=1, D=2, SD=3.

Sum the scores for the 10 items.

The higher the score, the higher the self perceived competence.

Appendix VI

Extroversion (Big Five Factor Markers)

Instructions: Below is a list of statements dealing with your general feelings about yourself. If you strongly agree, circle SA. If you agree with the statement, circle A. If you disagree, circle D. If you strongly disagree, circle SD

1.	I make friends easily	SA	A	D	SD
2.	I am the life of the party	SA	A	D	SD
3.	I know how to captivate people	SA	A	D	SD
4. *	I keep in the background	SA	A	D	SD
5. *	I would describe my experiences as somewhat dull	SA	A	D	SD
6.*	I don't want to draw attention to myself	SA	A	D	SD
7.	I am skilled in handling situations	SA	A	D	SD
8.*	I have little to say	SA	A	D	SD
9.*	I don't talk a lot	SA	A	D	SD
10.	I feel comfortable around people	SA	A	D	SD

Scoring Instructions *(NB: not seen by panellists)*

SA=3, A=2, D=1, SD=0.

Items with an asterisk are reverse scored, that is, SA=0, A=1, D=2, SD=3.

Sum the scores for the 10 items.

The higher the score, the higher the self perceived extroversion.

Appendix VII

Rosenberg Self-Esteem Scale (Rosenberg, 1965).

Instructions: Below is a list of statements dealing with your general feelings about yourself. If you strongly agree, circle SA. If you agree with the statement, circle A. If you disagree, circle D. If you strongly disagree, circle SD.

1.	On the whole, I am satisfied with myself.	SA	A	D	SD
2.*	At times, I think I am no good at all.	SA	A	D	SD
3.	I feel that I have a number of good qualities.	SA	A	D	SD
4.	I am able to do things as well as most other people.	SA	A	D	SD
5.*	I feel I do not have much to be proud of.	SA	A	D	SD
6.*	I certainly feel useless at times.	SA	A	D	SD
7.	I feel that I'm a person of worth, at least on an equal plane with others.	SA	A	D	SD
8.*	I wish I could have more respect for myself.	SA	A	D	SD
9.*	All in all, I am inclined to feel that I am a failure.	SA	A	D	SD
10.	I take a positive attitude toward myself.	SA	A	D	SD

Scoring Instructions *(NB: not seen by panellists)*

SA=3, A=2, D=1, SD=0.

Items with an asterisk are reverse scored, that is, SA=0, A=1, D=2, SD=3.

Sum the scores for the 10 items.

The higher the score, the higher the self esteem.

Appendix VIII

Rosenberg Self-Esteem Scale (Rosenberg, 1965).

Instructions: Below is a list of statements dealing with your general feelings about yourself. If you strongly agree, circle SA. If you agree with the statement, circle A. If you disagree, circle D. If you strongly disagree, circle SD.

1.	On the whole, I am satisfied with myself.	SA	A	D	SD
2.*	At times, I think I am no good at all.	SA	A	D	SD
3.	I feel that I have a number of good qualities.	SA	A	D	SD
4.	I am able to do things as well as most other people.	SA	A	D	SD
5.*	I feel I do not have much to be proud of.	SA	A	D	SD
6.*	I certainly feel useless at times.	SA	A	D	SD
7.	I feel that I'm a person of worth, at least on an equal plane with others.	SA	A	D	SD
8.*	I wish I could have more respect for myself.	SA	A	D	SD
9.*	All in all, I am inclined to feel that I am a failure.	SA	A	D	SD
10.	I take a positive attitude toward myself.	SA	A	D	SD

Scoring Instructions *(NB: not seen by panellists)*

SA=3, A=2, D=1, SD=0.

Items with an asterisk are reverse scored, that is, SA=0, A=1, D=2, SD=3.

Sum the scores for the 10 items.

Appendix IX

Physical Activity Readiness

Questionnaire - PAR-Q
(Revised - July 2007)

PAR-Q & YOU

(A Questionnaire for People Aged 15 to 69)

Regular physical activity is fun and healthy, and increasingly more people are starting to become more active every day. Being more active is very safe for most people. However, some people should check with their doctor before they start becoming much more physically active.

If you are planning to become much more physically active than you are now, start by answering the seven questions in the box below. If you are between the ages of 15 and 69, the PAR-Q will tell you if you should check with your doctor before you start. If you are over 69 years of age, and you are not used to being very active, check with your doctor.

Common sense is your best guide when you answer these questions. Please read the questions carefully and answer each one honestly : Check YES or NO

	Yes	No
Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor?		
Do you feel pain in your chest when you do physical activity?		
In the past month, have you had chest pain when you were not doing physical activity?		
Do you lose your balance because of dizziness or do you ever lose consciousness?		
Do you have a bone or joint problem (for example, back, knee or hip) that could be made worse by a change in your physical activity?		
Is your doctor currently prescribing drugs (for example, water pills) for your blood pressure or heart condition?		
Do you know of any other reason why you should not do physical activity?		

If you answered YES to any of these questions:

Talk with your doctor by phone or in person BEFORE you start becoming much more physically active or BEFORE you have a fitness appraisal. Tell your doctor about the PAR-Q and which questions you answered YES.

You may be able to do any activity you want -as long as you start slowly and build up gradually. Or, you may need to restrict your activities to those which are safe for you. Talk with your doctor about the kinds of activities you wish to participate in and follow his/her advice.
Find out which community programs are safe and helpful for you.

NO to all questions:

If you answered NO honestly to all PAR-Q questions, you can be reasonably sure that you can :

- start becoming much more physically active - begin slowly and build up gradually. This is the safest and easiest way to go.
- take part in a fitness appraisal – this is an excellent way to determine your basic fitness so that you can plan the best way for you to live actively. It is also highly recommended that you have your blood pressure evaluated. If your reading is over 144/94, talk with your doctor before you start becoming much more physically active.

DELAY BECOMING MUCH MORE ACTIVE:

- if you are not feeling well because of a temporary illness such as a cold or a fever - wait until you feel better; or
- if you are or may be pregnant - talk to your doctor before you start becoming more active.

Please note: If your health changes so that you then answer "YES" to any of the above questions, tell your fitness or health professional. Ask whether you should change your physical activity plan.

Informed Use of the PAR-Q: The Canadian Society for Exercise Physiology, Health Canada, and their agents assume no liability for persons who undertake physical activity, and if in doubt after completing this questionnaire, consult your doctor prior to physical activity.

NOTE: If the PAR-Q is being given to a person before he or she participates in a physical activity program or a fitness appraisal, this section may be used for legal or administrative purposes.

"I have read, understood and completed this questionnaire. Any questions I had were answered to my full satisfaction."

Signature: Identity Document No.:

Name: Date:

Signature of Parent or Guardian: Witness:
(for participants under the age of majority)

Note:

1.The information provided on this form will only be used for the application for use of Leisure and Cultural Services Department's Fitness Rooms and enrolment of recreation and sports activities. For correction of or access to personal data collected by means of this form, please contact staff of the enrollment counter/district.

2.If you answer "yes" to one or more questions in the "PAR-Q & YOU", your physical condition may not be suitable for taking part in the activity concerned. For safety's sake, you should consult a doctor in advance and produce a medical certificate upon enrolment or hire of fitness equipment to prove that you are physically fit for taking part in the activity. If you fail to produce a medical certificate, you must submit the completed Declaration upon enrolment or hire of fitness equipment.

This physical activity clearance is valid for a maximum of 12 months from the date it is completed and becomes invalid if your condition changes so that you would answer YES to any of the seven questions.

Appendix X

Linking odour chemistry and human behaviour

PARTICIPANT NUMBER:_____ DATE: 21 Oct 2009

Thank you for taking part in this study, the information provided will be used only in the data analysis and will be completely confidential. Some questions in the Relationships section are of a personal nature – you are free not to answer these. However if you do, it will help us explore the research question, and all answers will be treated with utmost confidentiality – they will be seen only by the researchers Patrizia D Ettorre and Alice Murray. Once all data are collected, we only use the participant code number and delete your name from our database.

On the following pages, there are phrases describing people's behaviours. Please use the rating scale below to describe how accurately each statement describes *you*. Describe yourself as you generally are now, not as you wish to be in the future. Describe yourself as you honestly see yourself, in relation to other people you know of the same sex as you are, and roughly your same age. Again, so that you can describe yourself in an honest manner, your responses will be kept in absolute confidence. Please read each statement carefully, and indicate how much you agree with each of the following statements by ticking the corresponding box.

Statement	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly agree
1. I dislike looking at myself in the mirror.					
2. I attract attention from the opposite sex.					
3. I have a pleasing physique.					
4. I like to look at myself in the mirror.					
5. I like to look at					

my body.					
6. I like to show off my body.					
7. I am considered attractive by others.					
8. I don't consider myself attractive.					
9. I dislike looking at my body.					
10. I let others make the decisions.					
11. I want to be in charge.					
12. I say what I think.					
13. I let myself be pushed around.					
14. I am not afraid of providing criticism.					
15. I take control of things.					
16. I can take strong measures.					
17. I take charge.					
18. I wait for others to lead the way.					
19. I never challenge things.					
20. Try to surpass others'					

accomplishments.					
21. I try to outdo others.					
22. I hate to seem pushy					
23. I am quick to correct others.					
24. I impose my will on others.					
25. I demand explanations from others.					
26. I want to control the conversation.					
27. I am not afraid of providing criticism.					
28. I challenge others' points of view.					
29. I lay down the law to others.					
Statement	**1** **Strongly disagree**	**2** **Disagree**	**3** **Neither agree nor disagree**	**4** **Agree**	**5** **Strongly agree**
30. I put people under pressure.					
31. I have taken frequent stands in the face of strong opposition.					

32. I avoid dealing with uncomfortable emotions.					
33. I don't hesitate to express an unpopular opinion					
34. I call for action while others talk.					
35. I do not stand up for my beliefs.					
36. I avoid dealing with awkward situations.					
37. I can face my fears.					
38. I don't speak my mind freely when there might be negative results.					
39. I speak up in protest when I hear someone say mean things.					
40. I am a brave person.					
41. I like having authority over others.					
42. I see myself as a good leader.					
43. I wait for others to lead the way.					
44. I want to be in					

charge.					
45. I am not highly motivated to succeed.					
46. I find it easy to manipulate others.					
47. I dislike having authority over others.					
48. I dislike taking responsibility for making decisions.					
49. I try to lead others.					
50. I have a strong need for power.					

Here are a number of personality traits that may or may not apply to you. Please tick in the column according to the extent to which you agree or disagree with the statement "I see myself as .....". You should rate the extent to which the **pair** of statements applies to you, even if one characteristic applies more strongly than the other.

I see myself as…..

	1 Disagree strongly	2 Disagree moderately	3 Disagree a little	4 Neither agree nor disagree	5 Agree a little	6 Agree moderately	7 Agree strongly
51. Extraverted, enthusiastic							
52. Critical, quarrelsome							
53.Dependable, self-disciplined							

54. Anxious, easily upset							
55. Open to new experiences, complex							
56. Reserved, quiet							
57. Sympathetic, warm							
58. Disorganised, careless							
59. Calm, emotionally stable							
60. Conventional, uncreative							

How much do you agree with the following statements?

61. I think I am facially very attractive. (*Please circle a number on this scale*)

1	2	3	4	5	6	7
Strongly disagree			Neither agree or disagree			Strongly agree

62. I think I am physically very attractive (*NB all physical features except your face*).

1	2	3	4	5	6	7
Strongly disagree			Neither agree or disagree			Strongly agree

63. Overall, I think I am very attractive (*both face and body*).

1	2	3	4	5	6	7
Strongly disagree			Neither agree or disagree			Strongly agree

64. I think I look very masculine facially

1	2	3	4	5	6	7
Strongly disagree			Neither agree or disagree			Strongly agree

65. I think I look very masculine physically.

1	2	3	4	5	6	7
Strongly disagree			Neither agree or disagree			Strongly agree

66. I think I look very masculine overall?

1	2	3	4	5	6	7
Strongly disagree			Neither agree or disagree			Strongly agree

About relationships...

67. Are you currently in a relationship with someone? (*tick*) Yes ☐ No ☐

68 If yes, for how long? (years/months) _____

69. How many relationships have you had in the past 5 years? Here we define a relationship as lasting more than 1 month. _____

70. Not including any current relationship, how long did your last relationship last (years, months)? _____

71. Are you…. Heterosexual? ☐ Homosexual? ☐ Bisexual? ☐

72. Please write your date of birth: _____

73. Are you... Right-handed Left-handed Ambidextrous (*circle one*)

74. Have you ever broken any fingers? *If so, please tick which.*

FINGER	LEFT HAND	RIGHT HAND
Little finger		
Ring finger		
Middle finger		
Index finger		
Thumb		

Some questions about the past few days….

75. Please tick below when you exercised (or otherwise raised a sweat)

Day/time	**Tick below where applicable**
Sunday morning (up to 12pm)	
Sunday afternoon (12pm – 6pm)	
Sunday evening (6pm – bedtime)	
Monday morning before sampling	
Monday morning after sampling	
Monday afternoon (12pm – 6pm)	
Monday evening (6pm – bedtime)	
Tuesday morning before sampling	
Tuesday morning after sampling	
Tuesday afternoon (12pm – 6pm)	
Tuesday evening (6pm – bedtime)	
Wednesday (before sampling)	

76. Please tick below when you showered/bathed

Day/time	**Tick below where applicable**
Sunday morning (up to 12pm)	
Sunday afternoon (12pm – 6pm)	
Sunday evening (6pm – bedtime)	
Monday morning before sampling	
Monday morning after sampling	
Monday afternoon (12pm – 6pm)	
Monday evening (6pm – bedtime)	
Tuesday morning before sampling	
Tuesday morning after sampling	
Tuesday afternoon (12pm – 6pm)	
Tuesday evening (6pm – bedtime)	
Wednesday (before sampling)	

77. In the table **above**, please also write a "P" whenever you may have used a perfumed product (deodorant, anti-perspirant) etc that may have reached your armpits.

78. Again in the table **above**, please write N whenever you used the unperfumed soap provided.
79. Please describe your alcoholic intake during the last few days.

Day	**Write below what you have drunk and how much**
Sunday	
Monday	
Tuesday	

Things that might affect your shirt-wearing:

80. Approximately how many hours did you spend in the t-shirt on the two nights. *For example, if you went to bed at 11 on Sun night and got up at 7, write "8"*

Monday night _____
Tuesday night _____

81. Do you smoke? Yes ☐ No ☐

82. Is it possible that the shirt you wore might have been contaminated by tobacco smoke – eg by a flatmate, yourself? Yes ☐ No ☐

83. On the scale of 1-7 below, please indicate the level of stress you may be feeling (eg through pressures of family, work) *please circle a number*

1	2	3	4	5	6	7
Very calm						Very stressed

84. Did you eat any of these foods? *Please tick if you did, leave blank if not*

Type of food	Monday	Tuesday
Raw Onion		
Lots of cooked onion		
Garlic		
Chilli		
Pepperoni		
Curry		
Strong spices or herbs		
Strong or smelly cheeses		
Cabbage		
Celery		
Asparagus		
Lamb		
Yoghurt		

85. Are you vegetarian? Yes ☐ No ☐

86. Are you a vegan? Yes ☐ No ☐

87. Did you sleep alone? (*circle as appropriate*) ☐ ☐

on the first night you wore the shirt	Yes	No
on the second night you wore the shirt	☐	☐

Appendix XI

Body odour and pheromone expt (March 2012)

Participant number _____

Your age: _____

Below is a set of phrases. Please use the scale below to describe how accurately each statement describes you (as you are now, not as you wish to be…). Describe yourself as you honestly see yourself, compared with people you know of the same sex/age. Indicate how much you agree with each of the following statements by ticking the corresponding box.

Statement	1 Strongly disagree	2 Disagree	3 Neither agree nor disagree	4 Agree	5 Strongly agree
I dislike looking at myself in the mirror					
I attract attention from the opposite sex					
I have a pleasing physique					
I like to look at myself in the mirror					
I like to look at my body					
I like to show off my body					
I am considered attractive by others					
I don't consider myself attractive					
I dislike looking at my body					
On the whole, I am satisfied					

with myself					
At times, I think I am no good at all					
I feel I have a number of good qualities					
I am able to do things as well as most other people					
I feel I do not have much to be proud of					
I certainly feel useless at times					
I feel that I'm a person of worth, at least on an equal plane with others					
I wish I could have more respect for myself					
All in all, I am inclined to feel that I am a failure.					
I take a positive attitude toward myself.					
I try to surpass others' accomplishments					
I try to outdo others					
I am quick to correct others					
I impose my will on others					
I demand explanations from others					
I want to control the conversation					
I am not afraid of providing criticism					
I challenge others' points of					

view					
I lay down the law to others					
I put people under pressure					
I hate to seem pushy					

PTO

Compared to others of the same sex and age….

1. How attractive do you consider your own face to be? *Please tick*

Very unattract	Unattrac	Neutra	Attracti	Very Attracti

2. **How attractive do you consider your own body to be?** *Please tick*

Very unattract	Unattrac	Neutra	Attracti	Very Attracti

3. **How would you best describe your appearance overall?** *Please tick*

Very unattract	Unattrac	Neutra	Attracti	Very Attracti

4. Are you currently in a relationship? YES / NO (*please circle*)

5. Would you describe yourself as predominantly heterosexual? Yes / No (*please circle*)

6. Smoking may influence your sense of smell. Do you smoke? Yes / No (*please circle*)

7. How much do you agree or disagree with the following statements? (*please tick*)

	Strongly disagree	Disagree	Neither Agree nor disagree	Agree	Strongly agree
Sex without love is OK					
I can imagine myself being comfortable and e "casual" sex with different partners					
I do *not* want to have sex with a person until that we will have a long-term, seriou relationship					

WOMEN ONLY

8. **Are you currently using a form of hormonal contraception?** (*please circle*)

No Combined pill Progesterone only pill Yes, another kind

9. If you answered 'No' to Q8: Women's sensitivity to different smells varies across the month. Please give the date of the first day of your last period (*use the calendar available if you need*)_____